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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME XLIX.

WOODS HOLE, MASS.

JULY TO DECEMBER, 1925

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

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I. TRUSTEES

(AS OF AUGUST 12, 1924).

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
LAWRASON RIGGS, JR., *Treasurer*, 25 Broad Street, New York City.
GARY N. CALKINS, *Clerk of the Corporation*, and *Secretary of the Board of Trustees*, Columbia University.

EMERITUS

CORNELIA M. CLAPP, Mount Holyoke College.

TO SERVE UNTIL 1928

H. H. DONALDSON, Wistar Institute of Anatomy and Biology.
W. E. GARREY, Tulane University.

Caswell Grave, Washington University.
M. J. Greenman, Wistar Institute of Anatomy and Biology.
R. A. Harper, Columbia University.
A. P. Mathews, The University of Cincinnati.
G. H. Parker, Harvard University.
C. R. Stockard, Cornell University Medical College.

TO SERVE UNTIL 1927

H. C. Bumpus, Brown University.
W. C. Curtis, University of Missouri.
George T. Moore, Missouri Botanical Garden, St. Louis.
W. J. V. Osterhout, Rockefeller Institute for Medical Research.
J. R. Schramm, Cornell University.
William M. Wheeler, Bussey Institution, Harvard University.
Lorande L. Woodruff, Yale University.

TO SERVE UNTIL 1926

E. G. Conklin, Princeton University.
Otto C. Glaser, Amherst College.
Ross G. Harrison, Yale University.
H. S. Jennings, Johns Hopkins University.
F. P. Knowlton, Syracuse University.
M. M. Metcalf, Oberlin, Ohio.
William Patten, Dartmouth College.
W. B. Scott, Princeton University.

TO SERVE UNTIL 1925

C. R. Crane, New York City, *President of the Corporation.*
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
E. P. Lyon, University of Minnesota.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, Columbia University.
D. H. Tennent, Bryn Mawr College.
E. B. Wilson, Columbia University.

Executive Committee of the Board of Trustees.

Frank R. Lillie, *Ex. off. Chairman.*
Gilman A. Drew, *Ex. off.*
Ivey F. Lewis, to serve until 1927.
E. G. Conklin, to serve until 1926.
W. E. Garrey, to serve until 1925.

THE LIBRARY COMMITTEE.

C. E. McCLUNG, Chairman, University of Pennsylvania.
M. M. METCALF, National Research Council.
J. R. SCHRAMM, Cornell University.
E. E. JUST, Howard University.
R. A. BUDINGTON, Oberlin College.

II. ACT OF INCORPORATION.

No. 3170

COMMONWEALTH OF MASSACHUSETTS.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

[SEAL] HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee of finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER.[1]

HARVEY S. CHASE & COMPANY
Certified Public Accountants
84 State Street, Boston.

February 9, 1925.

MR. LAWRASON RIGGS, JR.,
25 Broad Street,
New York.

Dear Sir: We have audited the accounts of the Marine Biological Laboratory for the year ended December 31, 1924 and report thereon in the following exhibits and schedules, together with our comments, viz:

Exhibit A—Balance-Sheet, December 31, 1924.
Exhibit B—Income-and-Expense, Year ended December 31, 1924.
  Schedule I—Securities held by Central Union Trust Company of New York as Trustees under Agreement dated January 22, 1924.
  Schedule II—Lucretia Crocker Fund, December 31, 1924.

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[1] Schedules IV and V are not included in this report. The complete audit is on file at the Laboratory, and may be examined by any member.

Schedule III—Cash Receipts and Payments, Reserve and Library Funds, January 8, 1924 to December 31, 1924.

Schedule IV—Land, Buildings, and Equipment, December 31, 1924.

Schedule V—Supply Department Income-and-Expense Account, Year ended December 31, 1924.

During the year 1924 the Laboratory received endowment fund gifts amounting to $905,000.00 and a building fund gift of $500,000.00.

The receipt of these large gifts made it advisable to change the arrangement of the balance-sheet and we have, therefore, set it up in the form that has been generally adopted by larger endowed institutions. We have also regrouped the items in the income-and-expense statement—Exhibit B.

We certify that the balance-sheet and income-and-expense statement shown in Exhibits A and B in our opinion set forth correctly the financial condition of the Marine Biological Laboratory at December 31, 1924, and the results of its operations for the year ended on that date.

Very respectfully,
Harvey S. Chase & Company,
Certified Public Accountants.

Exhibit A

Marine Biological Laboratory Balance-Sheet, December 31, 1924.

Assets.

Endowment Fund Assets:			
Securities in Hands of Trustee—Schedule I		$905,097.50	
Cash in Hands of Trustee		12.00	
		$905,109.50	
Lucretia Crocker Fund Assets,			
Securities—Schedule II	$ 4,005.17		
Cash—Schedule II	427.19	4,432.36	$ 909,541.86
Plant Assets:			
New Laboratory Building,			
Expenditures to December 31, 1924		$452,106.14	
Cash in Building Fund		56,615.53	
Total (See Contra)		$508,721.67	

Other Plant Assets—Schedule IV,			
Land	$102,094.89		
Buildings	236,126.82		
Equipment	108,837.49		
	$447,059.20		
Less Reserve for Depreciation	69,768.47	377,290.73	886,012.40
Current Assets:			
Cash,			
In New York Bank	$ 13,821.67		
In Hands of Trustee	2,200.00		
In Falmouth Bank	4,228.95		
Petty Cash	500.00	$ 20,750.62	
Accounts—Receivable		19,586.38	
Inventories,			
Supply Department	$ 24,558.41		
Biological Bulletin	3,483.21	28,041.62	
Investments,			
Gansett Property Account	$ 7,993.23		
Stock in General Biological Supply House, Inc.	12,700.00	20,693.23	
Prepaid Insurance		1,834.71	
Items in Suspense		1,313.32	92,219.88
			$1,887,774.14

Liabilities.

Endowment Funds:		
Friendship Fund, Inc.	$405,000.00	
John D. Rockefeller, Jr.	400,000.00	
Carnegie Corporation	100,000.00	
Gain on Sale of Securities during Year	109.50	
	$905,109.50	
Lucretia Crocker Fund	4,432.36	$ 909,541.86
Plant Funds:		
Rockefeller Foundation Contribution for New Laboratory Building	$500,000.00	
Interest Received on same during Year	8,721.67	
	$508,721.67	
Other Investment in Plant from Gifts and from Current Funds	377,290.73	
		886,012.40
Current Liabilities and Surplus:		
Mortgage on Gansett Property	$ 8,182.01	
Accounts-Payable	398.85	
Surplus,		

Balance, January 1, 1924	$427,309.99		
Add,			
Proceeds of Library and Reserve Fund Securities	9,715.24		
Balance of Income for Year—Exhibit B	23,904.52		
	$460,929.75		
Deduct,			
Book Value of Plant Assets transferred to "Plant Funds" above	377,290.73	83,639.02	
			92,219.88
			$1,887,774.14

EXHIBIT B

MARINE BIOLOGICAL LABORATORY, INCOME AND EXPENSE, FOR YEAR ENDED DECEMBER 31, 1924.

	Total		Net	
	Expense	Income	Expense	Income
Income, Endowment Fund		$ 33,656.58		$33,656.58
Donation for Expenses:				
Friendship Fund, Inc.		4,880.00		4,880.00
Others		25.00		25.00
Instruction	$ 7,516.39	10,095.00		2,578.61
Research	3,125.90	6,875.00		3,749.10
Biological Bulletin and Membership Dues	5,127.14	4,943.79	$ 183.35	
Supply Department, Schedule V	48,379.66	56,645.08		8,265.42
Mess Department	24,948.95	26,776.71		1,827.76
Dormitories Department	3,282.85	3,492.25		209.40
Interest and Depreciation charged to above Three Depts.	10,221.99			10,221.99
Dividends on Stock in General Biological Supply House, Inc.		976.00		976.00
Rent of Microscopes		319.50		319.50
Interest on Bank Deposits		103.54		103.54
Newman Cottage	122.85	150.00		27.15
Sundry Income		84.98		84.98
Maintenance of Plant:				
Maintenance of Buildings and Grounds	$ 6,121.19			
New Laboratory Expense	4,567.66			
Chemical Department	2,776.50			
Library Expenses	2,563.56			
Sundry Expenses	1,322.32			

Carpenter Department	1,080.28			
Dexter House, Special	971.35			
Truck Expenses	567.06			
Bar Neck Property	375.00			
Pumping Station	258.95			
Evening Lectures	111.41			
Janitor's House	11.77		20,727.05	
General Expenses:				
Administrative Expense	$ 10,981.15			
Endowment Fund, Trustee	787.50			
Interest on Loans	386.84			
Bad Debts	346.51		12,502.00	
Reserve for Depreciation	$ 9,608.11		9,608.11	
		$149,023.43		$66,925.03
	$125,118.91	125,118.91	$43,020.51	43,020.51
Balance of Income carried to Current Surplus—Exhibit A		$ 23,904.52		$23,904.52

SCHEDULE I

MARINE BIOLOGICAL LABORATORY SCHEDULE OF SECURITIES HELD BY CENTRAL UNION TRUST COMPANY OF NEW YORK AS TRUSTEE UNDER AGREEMENT DATED JANUARY 22, 1924.

DECEMBER 31, 1924.

Bonds.

Par Value		Cost
$ 25,000.	Chesapeake & Potomac Telephone Company of Virginia, 1st 30 Year S/F "A" 5s, due May 1, 1943; interest May and November 1	$ 23,812.50
*25,000.	Great Western Power Company, 1st Mortgage S/F 40 Year 5s, due July 1, 1946; interest January and July 1	25,000.00
*50,000.	Government of the Dominion of Canada, 30 Year 5s due May 1, 1952, interest May and November 1	50,000.00
25,000.	Home Long Distance Telephone Company, 1st S/F 20 Year 5s, due January 2, 1932; interest January and July 2	24,031.25
*25,000.	Joint Stock Farm Loan Bonds, The Lincoln Joint Stock Land Bank of Lincoln, Nebraska, 5s due November 1, 1938; interest May and November 1	25,000.00
*50,000.	Illinois Bell Telephone Company, 1st and Refunding Mortgage "A" 5s due June 1, 1956; interest June and December 1	50,000.00
*10,000.	Indiana Steel Company, 1st Mortgage 5s due May 1, 1952, interest May and November 1	10,000.00
*25,000.	Indianapolis Gas Company of Indianapolis, Indiana, 1st Consolidated Mortgage, 5s due April 1, 1952; interest April and October 1	25,000.00

25,000.	Lehigh Valley Harbor Terminal Railway Company, 1st 5s due February 1, 1954; interest February and August 1.	24,187.50
50,000.	Mortgage Bond Company of New York, Series No. 4, 6s due November 1, 1933; interest May and November 1..	49,500.00
*10,000.	Nashville Florence & Sheffield Railway Company, 1st Mortgage 5s due August 1, 1937; interest February and August 1	10,000.00
*8,000.	New Castle Company, Delaware Highway Improvement 4½s due January 1, 1932; interest January and July 1..	8,000.00
*50,000.	National Tube Company, 1st Mortgage 5s due May 1, 1952; interest May and November 1	50,000.00
*10,000.	New Castle Company, Delaware Highway Improvement 5th Series 4½s due January 1, 1931; interest January and July 1	10,000.00
*10,000.	New Castle Company, Delaware Highway Improvement 5th Series 4½s due January 1, 1930; interest January and July 1	10,000.00
10,000.	Philadelphia, Baltimore and Washington Railroad Company, General "B" 5s due February 1, 1974; interest February and August 1	9,875.00
15,000.	Reading Company, General and Refunding "A" 4½s due January 1, 1997; interest January and July 1	13,403.75
*10,000.	Southern Railway Company, 1st Consolidated Mortgage 5s due July 1, 1994; interest January and July 1	10,000.00
*50,000.	State of Louisiana Port Commission Serial Canal 5s due July 1, 1958; interest January and July 1	50,000.00
*10,000.	Southern Public Utilities Company, 1st and ref. mortgage 30 Year 5s due July 1, 1943; interest January and July 1.	10,000.00
*12,000.	U. S. A. 2nd Liberty Loan 4¼s due November 15, 1942; interest May and November 15	12,000.00
*25,000.	Virginian Railway Company, 1st Mortgage, 50 Year "A" 5s due May 1, 1962; interest May and November 1	25,000.00
*25,000.	Waco, Texas, Sewerage Disposal Plant, 5s due January 1, 1948; interest January and July 1	25,000.00
25,000.	West Shore Railroad Company, Gtd. Registered 1st 4s due January 1, 2361; interest January and July 1	19,687.50

Real Estate Mortgages

30,000.	B/M Alva Realty Company, 1321/7 Amsterdam Avenue, 5½% due February 1, 1929, interest February and August 1	30,000.00
30,000.	B/M Alva Realty Company, 129 W. 56th Street, 5½% due January 28, 1927; interest January and July 28	30,000.00
50,000.	B/M Cordelia Realty Corporation, 47/51 E. 52nd Street, 5½% due December 5, 1928; interest June and December 1	50,000.00
50,000.	B/M S & L Bldg. Company, N. E. Corner 82nd Street and Broadway, 5½% due April 9, 1933; interest June and December 1	50,000.00

* Securities received from Friendship Fund, Inc., taken at Par Value. Total, $405,000.00.

50,000.	B/M M & H Friedel, 328/38 E. 46th Street, 5½% due January 23, 1929; interest January and July 23	50,000.00
50,000.	B/M Canebrake Realty Company, 708–12 Greenwich Street, 5½% due December 29, 1928; interest June and December 29	50,000.00
30,000.	B/M Spruce & William Street Realty Company, N. E. Corner Spruce and William Streets, 5½% due December 30, 1928; interest June and December 30	30,000.00
23,000.	B/M W. T. Slevin, 47 Murray Street, 5½% due August 1, 1926; interest February and August 1	23,000.00
22,600.	B/M Bertha Kahn, 594/6 Broadway, 5½% due March 11, 1929; interest March and September 11	22,600.00
$915,600.		
	Total, December 31, 1924, Exhibit A	$905,097.50

SCHEDULE II

MARINE BIOLOGICAL LABORATORY, LUCRETIA CROCKER FUND, DECEMBER 31, 1924.

Investments

18 shares Vermont & Massachusetts Railroad Company	$2,416.50
3 shares American Telephone & Telegraph Company	504.04
4 shares Boston Consolidated Gas Company, Preferred	420.58
3 shares General Electric Company	349.55
16½ shares General Electric Company Special @ $11.00	181.50
4½ shares General Electric Company Special (Received as Stock Dividend)	
1 share Boston Elevated Railway Company, Second Preferred	133.00
Total, December 31, 1924—Exhibit A	$4,005.17

Cash Transactions for Year

Cash on Hand, January 8, 1924		$ 373.78
Received from Sale of Liberty Loan and American Telephone & Telegraph Company Bonds:		
Cost, as shown in Schedule I of Audit Report for 1923	$494.75	
Gain on Sale	4.98	499.73
Received for Interest and Dividends		189.87
Received from Sale of Rights		3.56
		$1,066.94
Paid for Securities transferred from Reserve and Library Funds	$564.75	
Paid for 1924 Scholarship	75.00	639.75
Balance on Hand, December 31, 1924—Exhibit A		$ 427.19

Schedule III

Marine Biological Laboratory Cash Receipts and Payments Reserve and Library Funds January 8, 1924 to December 31, 1924.

Reserve Fund:		
Cash on Hand, January 8, 1924		$ 782.93
Received from Sale of Securities,		
Cost as shown in Schedule I of Audit Report for 1923	$5,895.76	
Gain on Sale	951.15	6,846.91
Received for 12 shares General Electric Company, Special, transferred to Crocker Fund		132.00
Received for Interest and Dividends		226.93
		$7,988.77
Transferred to Laboratory General Cash Account		$7,988.77
Library Fund:		
Cash on Hand, January 8, 1924		$ 20.13
Received from Sale of Securities,		
Cost as shown in Schedule I of Audit Report for 1923	$1,957.81	
Gain on Sale	348.01	2,305.82
Received for Securities transferred to Crocker Fund: Cost	$ 362.38	
Gain on Transfer	70.37	432.75
Received for Interest and Dividends		97.46
Received from Sale of Rights		10.65
		$2,866.81
Transferred to Laboratory General Cash Account		$2,866.81

The Reserve Fund and the Library Fund were, as shown above, closed out during the year by order of the Executive Committee, the proceeds of the Reserve Fund being used for the acquisition of new property, and the proceeds of the Library Fund being used for the purchase of books.

Respectfully submitted,
LAWRASON RIGGS, JR.,
Treasurer.

V. REPORT OF THE ASSISTANT LIBRARIAN DECEMBER 31, 1924.

Along with the start of a new library building made a year ago, there has been a steady effort on the part of the Library Staff to so adapt their activities that, with the least expenditure of money and effort, our acquisitions and our new arrangements should fall into ready adjustment with the new and enlarged quarters. Even before the beginning of the year of 1924, a special sum had, at the request of the Assistant Librarian and of the Library Committee, been granted for the purchase of back sets—a sum of $400.00, this entirely outside the appropriation for the years 1923 and 1924. At the same time $350.00 had been granted for the employment of a secretary for at least a part of the year 1924. A start in purchasing back sets of serials was therefore well begun by January 1, 1924 and on the first of May the services of a secretary-stenographer were secured. With this kind of help, the Assistant Librarian has felt that work of real value to the Library has gotten under way.

The Assistant Librarian was also authorized to anticipate the use of a sum of $4,000.00 that would be available before the expiration of the year 1924 from the accumulation of the endowment fund; this was used in the purchase of back sets in the early spring that would, a few months later, have been unavailable. These were German sets for sale in this country, not as yet affected by the great jump made in German prices. During the late spring, extensive orders were placed in England with Heffer, and still later information showed France and French sets to be the best sales available. Certain sets of American journals were ordered at this same time, at high prices, but only where sets were out of print and no lessening of price could ever be anticipated. A detailed listing of the sets ordered and come into our actual possession during the year 1924 will follow later with the statement of other acquisitions.

On September 20, the Secretary of the Executive Committee handed a statement to the Assistant Librarian authorizing a provisional budget of $6,500.00 for the year 1925 as well as the use of a further sum of something over $2,500.00 for the year

1924–25 from the sale of the Library Fund securities. These actually realized $2,866.86. The Assistant Librarian was further assured by this same statement of the Executive Committee that the secretary would be retained and that early in the year 1925 the salary for an assistant would be available, besides a small sum for "man labor," over and above the sums stated on the budget and the $4,000.00 above referred to as a special sum. Along with this definite backing, the Assistant Librarian was also assured that any reasonable distribution for the two years of 1924–25 expenditures could be mapped out that seemed wise. For this reason and because of the practicability of running the two years together the provisional distribution of the total sum for 1924–25 of about $15,000.00 ($1,750.00 having been appropriated for expenditures aside from salaries for 1924) has been made as follows: current subscriptions, $3,000.00; books, $1,300.00; binding $2,000.00; back sets, $8,000.00; express, $200.00; supplies, $500.00.

A feature as interesting as that of securing back sets of our current serials, is that of securing new current subscriptions. The sum for this purpose we propose to double in the year 1925 over 1924 which latter was $1,000.00, thus spending $3,000.00 for the two years. A list of those now already undertaken for the years 1924 and 1925 will be given later. The negotiation for new exchanges in current serials is being carried on with success most encouraging for the effort made. The new exchanges that have been secured for the BIOLOGICAL BULLETIN, already begun for the years 1924 and 1925, are also given below.

With the larger funds available in these two years a really generous increase in the amount spent for books seemed right, especially since our attitude of favoring serials over books will for obvious reasons continue. And there are many gaps in our reference books in both systematic and morphological works as well as strictly physico-chemical lines. Important works in each of these lines have been purchased as is seen in the summary. There have been many gifts of books during the year. Very especial appreciation we hope may be conveyed to the authors of books who have remembered the users of the Library; and to the publishers who have so wisely and generously seen the advantages that the Library offers for the spreading of news as to a good book.

These gifts have all been acknowledged personally and a full list follows at the end of the report.

With the anticipated increase in space and especially the anticipated assistance to be given by another member of the Library Staff, trained in science and language, a special plea for reprints was sent at the end of December to each member of the following societies: American Physiological Society; American Society of Biological Chemists; American Society of Pharmacology and Experimental Therapeutics; American Society for Experimental Pathology; Ecological Society of America; Botanical Society of America; American Society of Zoölogists; American Society of Anatomists; American Society of Naturalists.

The return postal cards have shown how generous and cooperative the spirit of the scientist is. We feel great encouragement in regard to building up complete sets of reprints. We should have asked each author to send a bibliographic list of his reprints which could be filed in with such of his reprints as we have represented. This will have to be undertaken in the future. A count of the reprints acquired by the Library for 1924 will be deferred until the year 1925 and will be incorporated with the report for that year.

It gives me pleasure to express here my appreciation of Miss Deborah Lawrence's assistance in the Library since September 1st as Secretary. She gives every promise of accurate and business-like executive. Miss Frances Childs performed this same duty well during the summer, and would have been continued except that she undertook the summer work solely with the provision that she be released to pursue her studies in business training on September 1st. At this same time it is also a pleasure to announce that Miss Margaret Olmsted, Vassar, '24, with special training in physics and chemistry, as well as in French and German, will begin her duties as assistant in the Library on February 2nd, 1925. At this same time also I would greatly like to express my appreciation for the many suggestions that the Library Committee have given during the year. The sympathetic co-operation that each member has shown toward the work in the Library adds the authority as well as the balance of the scientist's point of view.

The Library now contains some 13,000 volumes, about eightninths of these being serial publications, mostly bound, and the remainder chiefly books. The reprints are about 9,800 with several hundreds besides these in our possession but so far uncatalogued. The number of currently received serials is about 332. Of these 132 are paid subscriptions and 200 are received by exchange for the Biological Bulletin, by gift or by loan. Forty-two subscriptions have been newly placed by purchase for the years 1924–25, and thirty-two newly secured by exchange. These are as follows:

Paid subscriptions: new in 1924: *Annales de l'Institut Pasteur; Archiv für experimentelle Pathologie und Pharmacologie; Chemical Reviews; Genetica; Ecology; Journal of the Chemical Society; Journal of Immunology; Journal of Medical Research; Journal of Scientific Instruments; Revue algologique; Revue générale d'histologie; Science Abstracts; Zeitschrift für Zellen- und Gewebelehre (Abteilung B. Zeitschrift für wissenschaftliche Biologie); Zeitschrift für vergleichende Physiologie (Abteilung C. Zeitschrift für wissenschaftliche Biologie).*

Paid subscriptions: new in 1925: *American Journal of Hygiene; Annals of Applied Biology; Annales de parasitologie humaine et comparé; Archives de morphologie générale et expérimentale; Archives de parasitologie; Brain; British Journal of Experimental Pathology; Centralblatt für Bakteriologie; Ergebnisse und Fortschritte der Zoologie; Flora; Allgemeine botanische Zeitschrift; Jahresberichte über die Fortschritte der Anatomie und Entwicklungsgeschichte; Journal de l'anatomie et de la physiologie; Journal de chimie physique; Journal de pharmacie et de chemie; Journal de physic; Journal of Botany; Journal of Experimental Pathology; Journal of Pathology and Bacteriology; Journal of the Washington Academy of Science; Kolloid-Zeitschrift: Beihefte; Quarterly Cumulative Index of Current Medical Literature; Ray Society Publications; Scientia; Tables annuelles de constantes; Virchow's Archiv für pathologische Anatomie und Physiologie und für klinische Medecin; Zeitschrift für Immunitats-Forschung; Zoologischer Bericht.*

Exchanges: new in 1924: *Acta hori bergiani; Annales scientifiques de l'Université de Jassey; Archives internationales de pharmacodynamie; Archives of internal medicine; Australian journal of experimental biology and medical sciences; Biologisches*

Centralblatt; Bulletin d'histologie appliquée à la physiologie, et à la pathologie, et de technique microscopique; Bulletin de l'Academie royale de Belgique; Bulletin de la société belge de biologie; Bulletin de la société royale des sciences médicales et naturelles de Bruxelles; Bulletin internationale de l'Academie des Sciences; Internationale Revue der gesamten Hydrobiologie und Hydrographie; Journal of Metabolic Research; Journal of the Linnean Society: Zoölogy; Monitore zoologico italiano; Nyt Magazin für Naturwidenskaberne; Osterreichische botanische Zeitschrift; Philippine journal of science; Recueil trauvaux botanique néerlandais; Publications of the Zoological Museum of Russia; Studies from the Plant Physiological Laboratory of Charles University, Prague; Transactions and Reports of the Royal Society of Australia; Transactions of the Connecticut Academy of Arts and Science.

Exchanges: new in 1925: *Acta phytochimica; Botanisches Archiv; Dansk Botanisk Tidskrift; Fermentforschung; Japanese Journal of Biochemistry; Medical Science: Abstracts and Reviews; Naturae Novitates; Ofversigt af Finska Vetenskaps-Societates, Förhanlingar*, and *Commentationes*, and *Acta; Zeitschrift für wissenschaftliche Zoologie.*

Besides currently received serials, there are on the shelves, sets, more or less complete, of serials to the number of about two hundred and fifty-three that have now ceased publication.

The following serials have been completed as sets by purchase as indicated, the expenditure for these having been about $4,251.80: *Acta horti Bergiani*, volumes 1–6, 1891–1919; *Annales de l'Institut Pasteur*, volumes 15–17; 24–38; *Annales des sciences naturelles: Zoologie*, series 4 to 9 filled in; (a few early volumes still missing); *Annals of Applied Biology*, volumes 1–10, 1914–24; *Annals of Botany*, 3–21; *l'Année biologique*, 1–21; *Annual Report of the Progress of Chemistry*, by volume 8; *Archives d'anatomie microscopique*, 1–16; *Archives de zoologie expérimentel et générale*, 32 scattered volumes; *Archives internationales de physiologie*, 1–14; *Archivio di farmacologia sperimentale et scienze affini*, 1–26; *Bibliographia Zoologica*, by volumes 30, 31, 32, and 34; (2 volumes still lacking); *Biochemische Zeitschrift*, 1–55, 1906–1913; 72–100, 1916–1919; *British Journal of Experimental Pathology*, 1–5, 1920–24; *Bulletin de l'Institut Pasteur*, 1–17, 1903–1909; *Ecology*, 1–4, 1920–23; *Fauna und Flora des Golfes von Neapel*, monographs 26–35a, 1901–1923; *Folia Neurobiologica*, 2 and 3–12, 1909–17 (volume 1, 1908, still lacking); *Harvey Lectures*, 1–16, 1905–1922; *Journal de physiologie et de pathologie générale*, 1–15; 17 (volumes 16 and 18 still lacking); *Journal of immunology*, volumes 1–8; *Journal of Physical Chemistry*, volumes 1–24; *Journal of the American Chemical Society*, 32–42, 1910–1920; *Journal of the Chemical Society* (*London*), volumes 45–124, 1884–1923; *Nuova Notarisia*, volumes 1–28, 1890–1918; *Physical Review*, 1—series 2, 16, 1893–1920; *Proceedings of the Academy of Natural Sciences of Philadelphia*, 41 volumes scattered thoughout the set; *Proceedings of the Royal Society of London*, 45–75, 1890–1905; *Psychobiology*, 1–2; *Quarterly Cumulative Index of Current Medical Literature*, 1 and 5–9 (2–4 still lacking); *Quarterly Journal of Microscopical Science*, volumes 18 and 19; *Revue générale d'histologie*, 1–3, 1904–1911; *Science Abstracts*, 1–26, 1898–1923; *Zeitschrift für physikalische Chemie*, 1–104, 1887–1923; *Zentralblatt für Zoologie, Allgemeine und experimentelle Biologie*, 1–6, 1912–1918. Many odd volumes besides these have been secured and paid under this sum above mentioned.

Through exchange of back sets for the Biological Bulletin, these sets have been secured in 1924: *Annales scientifiques de l'Université de Jassey*, 1–12, 1900–1923; *Annuaire du museum zoologique de l'Academie Imperial des Sciences de la Russie*, 9–13, 1914–20; *Archives internationales de pharmacodynamie*, volumes 1–29; *Bulletin de la classe des sciences, l'Academie royale de Belgique*, series 5, volumes 5–9, 1919–23; *Fauna of Russia*, volumes 1914–19; *Monitore zoologico italiano*, 1907–1924; *Nouvelles archives du Museum d'Histoire Naturelle*, Paris, series 2, 1—series 5, 6, 1878–1914; *Nyt magazin für Naturvidenskaberne grumdlagt au den physiographiske Forening, Christiania*, volumes 46–60, 1909–1923; *Ofversigt af Finska Venteskaps-Societetens; Förhandlingar*, volumes 45–64; *Osterreichische botanische Zeitschrift*, volumes 71–74, 1922–24; through exchange of duplicates of the Museum of Comparative Zoölogy publications and of the Boston Society of Natural History publications, the Library has, this year, also, filled in nearly all the bad gaps that existed, so that these sets are now practically complete.

The more important year's purchases of books and sets of reference works as regarded from the view-point of cost, are as follows: *Vergleichende Anatomie des Nervensystems*, by C. U. Ariêns Kappers und Droogleever Fortuyn; Barnes and Heald: *Analytic key to mosses of the United States;* Berlese, A.: *Gli Insetti;* Daniel, John Franklin: *The Elasmobranch fishes;* De Toni: *Sylloge Algorum*, volume 6; Helmholtz: *Handbuch der physiologischen Optik;* Jellinek, K.: *Physikalische Chemie der homogenen und heterogenen Gasreaktionen;* Leitgeb, H.: *Untersuchungen uber die Lebermuse;* Morgan, T. H.: *Regeneration;* Morse, H. N.: *Investigations in Osmotic pressure; Ouvres* de Pasteur: *Dissymetrie moleculaire, Fermentations et generations dites spontanées, Études sur le vinaigre et sur le vin;* Parker and Haswell: *Text-book of Zoölogy* 3d edition; *Plankton Expedition: Ergebnisse der in der Atlantisch Ozean*, etc.; Sars: *Crustacea of Norway*, 1–3; *United States Catalogue of 1912–1917;* Wiesner, Julius von and Moeller, J.: *Die Rohstoffe des Pflanzenreiches;* Williams, L. W.: *Anatomy of the Common Squid;* Wolle, Francis: *Green Algae of the United States;* Wood: *Physical Optics.*

Through gifts we have received as follows: new current serials and to continue: *American Journal of Photography* (now issued as *Camera*), *British Journal of Photography*, *Journal of the Photographic Institute*, *Journal of the Optical Society of America, and Review of Scientific Instruments*, *Photographic journal of the Royal Society of London*, from Dr. Pond; *Public Health Report*, from the United States Health Service; *Monographs*, from Cold Spring Harbor; *Bulletin of the University of Wisconsin*, and *Wisconsin Studies in Science*, at the request of Professor Guyer.

From the publisher, Blakiston: Atwood, W. H.: *Problems, Projects, and Experiments in Biology;* and *Comparative Vertebrate Dissection;* Clark, A. J.: *Applied Pharmacology: Scientific Evidence for Therapeutic Active Drugs;* Hawk, Philip B.: *Practical Physiological Chemistry;* Palladin, *Plant Physiology;* Pratt, H. S.: *Land and Freshwater Vertebrate Animals of the United States;* from the publisher, Saunders: Howell: *Physiology;* Lusk, Graham: *Elements of the Science of Nutrition* (at the request of the author); Mallory and Wright: *Pathological Technique;* Wells, H. Gideon: *Chemical Pathology;* from the New Haven Yale University Press: Woodruff, L. L.: *Development of the Sciences* (at the request of the author); from the publisher, Wiley: Underhill, Frank P.: *Manual of Selective Biochemical Methods; San*

Francisco Bay Marine Piling Survey Reports, from the Committee; from the publisher, Macmillan: Smith, Gilbert M.: *A Textbook of General Botany;* from Newman, Horatio Hackett: *Outlines of General Zoölogy*, and *Evolution;* from the Cape Cod Chamber of Commerce: *Special Report on Population and Resources of Cape Cod;* from H. S. Jennings: *Life and Death, Heredity and Evolution in Unicellular Organisms;* from Dr. Pond: Fischer, Max: *Uber den Abbau von Chlorophyll und Blutforbstaff;* from Lusk, Graham: *Science of Nutrition;* from Fischer, M.: *Oedema;* from Lillie, R. S.: *Protoplasmic Action and Nervous Action;* from Mrs. Jacques Loeb: *Regeneration from a Physico-chemical Viewpoint;* from Price, Weston A.: *Dental Pathology;* through Professor Tower from the American Museum of Natural History, Dean, Bashford: *A Bibliography of Fishes;* from Loeb, Leo: *Edema;* from Winge, Herluf: *Pattedyr-Slaegter;* from Schroder, Paul: *Introduction to the Histology and Histopathology of the Nervous System;* from the National Research Council: Atwood and Johnson: *Marine Piling Investigations;* from Hyman, L. H.: *Laboratory Manual of Elementary Zoölogy;* from Wheeler, Wm. M.: *Social Life among the Insects;* Professor Wheeler sent to us several boxes of reprints and odd volumes and numbers of journals also, which we were glad to get. Mrs. Montgomery presented some twelve books to the Library.

VI. THE DIRECTOR'S REPORT.

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg to submit herewith a report of the thirty-seventh session of the Marine Biological Laboratory for the year 1924.

1. *Attendance.*—As stated in the annual report for 1923 it was decided to set definite numerical limits to each of the courses of instruction and this was accordingly done and acted on in 1924. The result was the admission of only 134 students selected from almost twice the number of applicants. These students were distributed among the classes as shown in the tabular view of attendance (p. 37), each class being registered up to its full authorized number. The selection from among the applicants is

made by the head of each course of instruction on a given date, May 15 of each year, on the basis of a detailed application and letters of recommendation.

The number of investigators was 194, the largest registration in the history of the Laboratory. It is interesting to note that 70 of these were "under instruction," that is for the most part graduate students from University laboratories. The opportunity that is afforded these students of comparing the teachings and methods of the various University laboratories is a great benefit educationally for them. The large attendance of workers of this age augurs well for the future of the Laboratory which must depend for its growth on the development and maintenance of the devotion to its interests of successive generations of scientific workers.

2. *Library.*—The report of the Acting Librarian is given in another place. It details the plans and the means available for enlargement of the library, which we should now set down as one of the principal ends to be emphasized in the development of the Laboratory during the next few years. Next to providing the materials for investigation, and facilities of modern and adequate type for the work of research itself, there is no material service that the Laboratory can render of greater importance than a thoroughly good working library. To that end a wing of the new building is devoted with stack space for 100,000 volumes. If this capacity be compared with the present possessions of the library, 13,000 bound volumes and about 10,000 reprints and pamphlets, it will be realized that our efforts on behalf of the library need not relax for lack of space for some years to come. The Acting Librarian is to be congratulated on getting an active policy of expansion into operation before the new quarters are entirely ready.

3. *The Report of the Treasurer* (pp. 5–12).—Particular interest attaches to the report of the Treasurer this year because it includes the new endowment funds and the results of building operations as well as the customary current accounts. The assets of the Laboratory are thus of quite a different order of magnitude at the end of 1924 as compared with previous years: $1,887,774.14 on December 31, 1924 as compared with $445,504.69 on December 31, 1923. Of this amount $909,541.86 represent

income-yielding securities, the remainder being in plant assets and current assets. An additional sum of at least $200,000.00 is available from the Friendship Fund for completion of the building and its equipment, which will increase the assets of the Laboratory to considerably over $2,000,000.00. The securities belonging to the "Reserve Fund" and the "Library Fund" of previous reports were sold during the year at considerable profit as compared with book value; the proceeds from the Reserve Fund were applied on the purchase of the Hubbard property, and the money from the Library Fund was used for purchase of books. These funds were accumulated from small savings of many years past for the purposes for which they were finally used. In the case of the "Reserve Fund" it was felt that the ownership of such a strategically placed piece of property as the Hubbard lot was worth much more to the Laboratory than the securities in the fund. The timeliness of conversion of the Library Fund into books requires no comment.

It will be observed that the current accounts of the year 1924 show a considerable surplus ($23,904.52) of income over expense (Exhibit B of Treasurer's report). The operations of the year were planned on the usual scale, without reference to the new endowment fund, which was, however, paid in and invested early in the year. The income, which was considerably increased by an unusually good showing of the Supply Department (Exhibit B) was thus largely in excess of requirements in spite of an additional grant to the Library during the year, and repayment of loans totaling $6,100.00. This surplus enables us to start the operation of the enlarged plant with a considerable degree of confidence. It should be said also that the estimates for the year 1925 show a slight excess of income over expense including some unusual items. The net impression of a study of the Treasurer's report is that the Laboratory has an adequate financial foundation for its present needs, but that the same rigid economy will be needed in the future as in the past.

4. *Building Progress.*—As stated in the last report the Rockefeller Foundation made an appropriation of $500,000.00 for the new building; Mr. C. R. Crane also gave a pledge on behalf of the Friendship Fund to contribute whatever additional money was needed, according to the architects' plans and specifications, for

completion and equipment of the building. When the bids for the building were received and the estimates for equipment added the total requirements were found to be $748,886.71 divided as follows:

General Construction, Geo. A. Fuller Co.	$478,120.00
Plumbing, C. H. Cronin, Inc.	54,860.92
Heating and ventilating, Cleghorn Co.	31,534.00
Electric work, Hixon Electric Co.	65,875.00
Elevator and book lift, Otis Elevator Co.	6,970.00
Architects and engineers' commissions	36,526.79
Furniture, equipment, etc.	65,000.00
Alterations in course of construction	10,000.00
	$748,886.71

The Friendship Fund accordingly appropriated a sum not in excess of $248,886.71, and not in excess of the cost of the items enumerated, above the contribution of $500,000.00 for building by the Rockefeller Foundation and interest on this fund. The Building Committee has made every effort to keep within the available funds with the result now clearly in sight of entirely avoiding the necessity of any additional payment for alterations in the course of construction and of some probable slight savings in other directions. The great care with which the plans and specifications were prepared by the architects, and especially Dr. Drew's constant oversight of the building operations and purchase of equipment have been notable factors in the achievement of this result.

It has certainly very rarely happened that an institution has received such signally generous treatment and confidence as the Marine Biological Laboratory has received from the donors of the building fund. The action of the Rockefeller Foundation in paying over their entire contribution in cash before building operations were begun amounted to an increase of their contribution by the interest received during construction. The action of the Friendship Fund in guaranteeing the completion and equipment of the Laboratory insured the hearty coöperation of the other contributors, created a notable spirit of confidence, and aroused our own organization to the highest state of morâle.

Ground was broken for the new building on March 20, 1924. No serious difficulties were experienced during the course of construction which now seems likely to be completed within a year from the date of beginning.

5. *Research Rooms.*—The charge for use of a research room during the summer session has remained at $100.00 since 1888. The Executive Committee reviewed the situation in 1924 and decided to make no change in the case of the wooden buildings, but in the case of the research rooms of the brick buildings, which are more than twice the size and much more amply supplied with fixtures, it seemed reasonable, and wise from the point of view of all members of our coöperative organization, to ask increased appropriations from the coöperating institutions using them. Accordingly a basic charge of $200.00 has been established for the summer season with the understanding that two persons may occupy such a room. This should be regarded as a first step towards an equitable arrangement that will permit the most efficient operation of the building for research purposes.

6. *Biological Bulletin.*—The forty-seventh volume of the BIOLOGICAL BULLETIN was completed in 1924. It had been maintained at about a constant size since its establishment, two volumes with about 650–700 pages per year. Lately in common with many other biological research journals it had been falling behind in promptness of publication so that about a year elapsed between receipt of manuscripts and their appearance in print. The cost of enlarging it so as to keep up with manuscripts offered was greater than the Laboratory could well afford. It has also become a very valuable medium of exchange for the library as stated in Mrs. Montgomery's report. It was therefore decided to enlarge it to 900 pages a year, about 75 pages per number and at the same time to raise the subscription price from $6.00 to $9.00 per year. Correspondingly the fees of members of the Corporation, who receive the BULLETIN without other subscription, were increased from $4.00 to $6.00 per year. The increase in size began with the first issue of 1925, and it is already apparent that the passage of a manuscript to publication will be reduced to at most six months, and it is hoped to a still shorter period. There has been no significant loss of subscriptions or of membership in the Corporation in consequence of the increased cost.

7. *Membership of the Board of Trustees.*—At the meeting of the

Corporation held August 12, 1924 Professor R. A. Harper of Columbia University was elected a member of the class of 1928, and the seven members of the class of 1924 were reëlected to serve until 1928.

The following resolution in memory of Jacques Loeb, member of the Laboratory since 1893 and of the Board of Trustees since 1897, was adopted by the Board of Trustees and by the Corporation of the Marine Biological Laboratory at their meetings on August 12, 1924:

> By the death of Dr. Jacques Loeb the world has lost one of the great men of his generation; Biology has lost one of the finest intellects that has ever been devoted to this branch of science; this Laboratory has lost one of its most eminent members. He stood out among his fellows as an investigator, as a teacher, and as a cultivated gentleman broadly interested in all aspects of nature and all the activities of men.
>
> As an investigator he was tireless in energy, ingenious in experimentation and exceptionally gifted in insight. He lost little time on false leads, but rather blazed his trail straight into new territory and attained his objective by simple and crucial experiments.
>
> He brought to his work a broad knowledge of related sciences. In the latest advances of chemistry and physics he was always informed, and his researches showed the breadth of his reading and the solid character of his scholarship.
>
> He had a poet's imagination held in check by practical and mathematical faculties of high order. Consequently his hypotheses were at once brilliant and founded on the best physico-chemical data available. He was ready to change his theories as new facts were discovered. He believed that explanations of phenomena must be expressible ultimately in mathematical terms. He profoundly influenced General Physiology not only by his theories and experimental results but also, quite as much, by the emphasis he laid upon the quantitative method.
>
> Professor Loeb came to Woods Hole first in 1892. The epoch-making discovery of artificial parthenogenesis was made at this laboratory. The antagonistic action of ions was demonstrated here. Many other researches which have influenced biological thought were carried on here and may be noted in the long list of papers and books which constitute his best monument. He founded the course in General Physiology at Woods Hole in 1893 and drected it for several years. He was a trustee from 1897 to his death. From 1910 he directed the branch laboratory of the Rockefeller Institute in coöperation with Marine Biological Laboratory.
>
> As a teacher he was enthusiastic and inspiring. His lectures were in advance of the times and full of suggestions for research. With his graduate students he was helpful and friendly and at the same time critical and stimulating. Those who were his students know how enormously they profited by his inspiring personality.
>
> As a man his interests were well-nigh universal. He found time to make himself familiar with a large literature. He enjoyed music and all the arts. He was interested in economics, sociology and government. His "Mechanistic Conception of Life" is an important contribution to Philosophy and Psychology.
>
> He was a kindly man. He was a lovable man. He hated war and all sham. He had an incisive sense of humor and loved a harmless joke. He was the center of any company and he had many friends.

This is the man we have lost. Woods Hole is not the same without him, but the inspiration of his work remains in our midst.

To his family we extend our sympathy and on the pages of our records we inscribe this memorial.

There are included as parts of this report the following addenda:

1. The Staff, 1924.
2. Investigators and Students, 1924.
3. Tabular View of Attendance.
4. Subscribing and Coöperating Institutions, 1924.
5. Evening Lectures, 1924.
6. Members of the Corporation.

1. THE STAFF, 1924.

FRANK R. LILLIE, *Director*, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

E. G. CONKLIN, Professor of Zoölogy, Princeton University.

CASWELL GRAVE, Professor of Zoölogy, Washington University.

H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.

FRANK R. LILLIE, Professor of Embryology, The University of Chicago.

C. E. McCLUNG, Professor of Zoölogy, University of Pennsylvania.

S. O. MAST, Professor of Zoölogy, Johns Hopkins University.

T. H. MORGAN, Professor of Experimental Zoölogy, Columbia University.

G. H. PARKER, Professor of Zoölogy, Harvard University.

E. B. WILSON, Professor of Zoölogy, Columbia University.

II. INSTRUCTION

ROBERT H. BOWEN, Assistant Professor of Zoölogy, Columbia University.

EDWARD F. ADOLPH, Instructor in General Physiology, University of Pittsburgh.

HORACE B. BAKER, Instructor in Zoölogy, University of Pennsylvania.

J. A. DAWSON, Instructor in Zoölogy, Harvard University.

S. W. GEISER, Assistant Professor of Zoölogy, Washington University.

CHRISTIANNA SMITH, Assistant Professor of Zoölogy, Mount Holyoke College.

B. H. WILLIER, Instructor in Zoölogy, The University of Chicago.

DONNELL B. YOUNG, Assistant Professor of Biology, Carleton College.

PROTOZOÖLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

LOUISE H. GREGORY, Associate Professor of Zoölogy, Columbia University.

FLORENCE DE L. LOWTHER, Instructor in Zoölogy, Barnard College.

EMBRYOLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

HUBERT B. GOODRICH, Professor of Biology, Wesleyan University.

BENJAMIN H. GRAVE, Professor of Biology, Wabash College.

CHARLES PACKARD, Assistant Professor, Peking Union Medical College.

HAROLD H. PLOUGH, Associate Professor of Biology, Amherst College.

CHARLES G. ROGERS, Professor of Comparative Physiology, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.

WALTER E. GARREY, Professor of Physiology, Tulane University.

RALPH S. LILLIE, Professor of General Physiology, The University of Chicago.

ALBERT P. MATHEWS, Professor of Biochemistry, The University of Cincinnati.

II. INSTRUCTION

MERKEL H. JACOBS, Professor of General Physiology, University of Pennsylvania.

WILLIAM R. AMBERSON, Assistant Professor of Physiology, University of Pennsylvania.

FRANK P. KNOWLTON, Professor of Physiology, Syracuse University.

REYNOLD A. SPAETH, Associate in Physiology, School of Hygiene and Public Health, Johns Hopkins University.

BOTANY

I. INVESTIGATION

S. C. BROOKS, Department of Public Health, Washington, D. C.

EDWARD M. EAST, Professor of Experimental Plant Morphology, Harvard University.

ROBERT A. HARPER, Professor of Botany, Columbia University.

E. NEWTON HARVEY, Professor of Physiology, Princeton University.

WINTHROP J. V. OSTERHOUT, Professor of Botany, Harvard University.

JACOB R. SCHRAMM, Professor of Botany, College of Agriculture, Cornell University.

II. INSTRUCTION

IVEY F. LEWIS, Professor of Biology, University of Virginia.

TRACY E. HAZEN, Associate Professor of Botany, Barnard College, Columbia University.

WILLIAM RANDOLPH TAYLOR, Assistant Professor of Botany, University of Pennsylvania.

WILLIAM H. WESTON, JR., Assistant Professor of Cryptogamic Botany, Harvard University. (Absent 1924.)

LIBRARY

——— ———, Librarian

PRISCILLA B. MONTGOMERY (Mrs. Thomas H. Montgomery, Jr.), Assistant Librarian, in charge.

CHEMICAL SUPPLIES

OLIVER S. STRONG, Associate Professor of Neurology, Columbia University, New York City, Chemist.

SUPPLY DEPARTMENT

George M. Gray, Curator.
Thomas M. Douthart, Assistant Curator.
John J. Veeder, Captain.
E. M. Lewis, Engineer.
A. W. Leathers, Head of Shipping Department.
Milton B. Gray, Collector.
A. M. Hilton, Collector.
J. McInnis, Collector.

F. M. MacNaught, Business Manager.
Herbert A. Hilton, Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS, 1924.

ZOÖLOGY—Independent Investigators.

Addison, William H. F., Professor of Histology and Embryology, University of Pennsylvania.
Appleton, Joseph L., Jr., Professor of Microbiology and Bacteriopathology, Evans Institute, University of Pennsylvania.
Azuma, Ryotaro, Physiological Laboratory, Tokio Imperial University.
Bagg, Halsey J., Associate in Biology, Cornell University Medical College.
Baker, Horace Burrington, Instructor, University of Pennsylvania.
Bigelow, Robert Payne, Professor of Zoölogy, Massachusetts Institute of Technology.
Bonnier, Gert, University of Stockholm, Stockholm, Sweden.
Boschma, Hilbrand, Chief Assistant in Zoolögy, University of Leyden.
Bowen, Robert H., Assistant Professor of Zoölogy, Columbia University.
Breitenbecher, J. K., Associate Professor of Zoölogy, University of Oklahoma.
Bridges, Calvin B., Research Assistant, Carnegie Institution of Washington.
Budington, Robert A. Professor of Zoölogy, Oberlin College.
Burns, Robert Kyle, Jr., Assistant in Zoölogy, Yale University.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Carothers, E. Eleanor, University of Pennsylvania.
Chambers, Robert, Professor of Microscopic Anatomy, Cornell University Medical College.
Clark, E. R., Professor of Anatomy, University of Georgia.
Clark, Eleanor L., Research worker, University of Georgia.
Conklin, Edwin Grant, Professor of Biology, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Dawson, James A., Instructor in Invertebrate Zoölogy, Harvard University.
Detwiler, S. R., Assistant Professor of Zoölogy, Harvard University.
Dolley, Wm. Lee, Jr., Professor of Biology, Randolph-Macon College.
Donaldson, Henry Herbert, Professor of Neurology, The Wistar Institute.
Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.
Glaser, Otto Charles, Professor of Biology, Amherst College.
Goldfarb, A. J., College of the City of New York.
Goodrich, Hubert Baker, Professor of Biology, Wesleyan University.

GRAVE, BENJAMIN H., Professor of Zoölogy, Wabash College.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
GREGORY, LOUISE HOYT, Associate Professor of Zoölogy, Barnard College, Columbia University.
HANCE, ROBERT T., University of Pennsylvania.
HEILBRUNN, LEWIS V., Assistant Professor of Zoölogy, University of Michigan.
HENTSCHEL, CHRISTOPHER CARL, King's College, London.
HESS, WALTER N., Johnston Scholar, Johns Hopkins University.
HIBBARD, HOPE, Associate Professor of Zoölogy, Elmira College.
HOGUE, MARY J., Professor of Bacteriology, North Carolina College for Women.
HUXLEY, JULIAN SORELL, Senior Demonstrator in Zoölogy, Oxford University.
HYMAN, LIBBIE HENRIETTA, Research Assistant, University of Chicago.
JENNINGS, HERBERT S., Professor of Zoölogy, Johns Hopkins University.
JUST, ERNEST E., Professor of Zoölogy, Howard University.
KINDRED, JAMES E., Assistant Professor of Histology and Embryology, University of Virginia.
KNOWER, HENRY MCELDERRY, Professor of Anatomy, University of Cincinnati.
KOMAI, TAKU, Assistant Professor of Zoölogy, Kyoto Imperial University.
LANCEFIELD, DONALD E., Assistant Professor in Zoölogy, Columbia University.
LANGE, MATHILDE M., Head of the Department of Biology, Wheaton College.
LILLIE, FRANK R., Chairman, Department of Zoölogy, University of Chicago.
LYNCH, RUTH STOCKING, Assistant in Graduate Zoölogy, Johns Hopkins University.
MCCLUNG, CLARENCE E., Director of Zoölogical Laboratory, University of Pennsylvania.
MARTIN, EARL A., Assistant Professor, College of the City of New York.
MAST, SAMUEL OTTMAR, Professor of Zoölogy, Johns Hopkins University.
MAVOR, JAMES W., Professor of Biology, Union College.
METCALF, MAYNARD M., 128 Forest St. Oberlin, Ohio.
METZ, CHARLES W., Investigator, Carnegie Institution, Cold Spring Harbor.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
MORGAN, LILIAN V., 409 W. 117th St., New York City.
MORRILL, CHARLES V., Associate Professor of Anatomy, Cornell University Medical College.
PACKARD, CHARLES, Associate in Zoölogy, Crocker Laboratory, Columbia University.
PAPANICOLAO, GEORGE N., Assistant Professor in Anatomy, Cornell University Medical College.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
RAND, HERBERT W., Associate Professor of Zoölogy, Harvard University.
RENYI, GEORGE S., Assistant Professor, University Medical College, Budapest.
ROGERS, CHARLES G., Professor of Comparative Physiology, Oberlin College.
SMITH, CHRISTIANNA, Assistant Professor, Mt. Holyoke College.
SPEIDEL, CARL CASKEY, Associate Professor of Anatomy, University of Virginia.
STARK, MARY B., Professor of Histology and Embryology, N. Y. Homœopathic Medical College.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.
STRONG, OLIVER S., Associate Professor of Neurology, Columbia University.
STURTEVANT, ALFRED H., Member of Staff, Carnegie Institution of Washington.
THOMPSON, IAN MACLAREN, Lecturer in Anatomy, McGill University.
WATSON, ISABELLE, Research Assistant, Carnegie Institution.

Wieman, Harry Lewis, Professor of Zoölogy, University of Cincinnati.
Willier, Benjamin H., Assistant Professor of Zoölogy, University of Chicago.
Wilson, Edmund B., Professor of Zoölogy, Columbia University.
Wood, Frank Elmer, Head of the Department of Biology, Illinois Wesleyan University.
Woodruff, Lorande L., Professor of Protozoölogy, Yale University.
Young, Donnell B., Assistant Professor of Biology, Carleton College.

ZOOLOGY—Beginning Investigators.

Adolph, Paul E., University of Pennsylvania.
Anson, Mortimer L., Cambridge University.
Becker, Elery R., Instructor, Princeton University.
Beerman, Herman, Washington University.
Beers, Charles Dale, Student, Johns Hopkins University.
Beers, Catherine V., Assistant Professor of Biology, University of Southern California.
Blake, Charles Henry, Student, Massachusetts Institute of Technology.
Bowen, Edith S., Instructor in Zoölogy, Wellesley College.
Brown, Alice L., Student and Assistant, Cornell University Medical College.
Cole, Kenneth, Instructor, Oberlin College.
Cook, Daniel, University of Cincinnati.
Cowperthwaite, Marion, Instructor, Mt. Holyoke College.
DeForest, David M., Union College.
Faber, Erwin F., Instructor in Anatomical Drawing, University of Pennsylvania.
Foster, Kendall W., Austin Teaching Fellow in Zoölogy, Harvard University.
Fry, Henry J., Instructor, Department of Biology, New York University Washington Square College.
Grand, Constantine G., Research, Cornell Medical College.
Grave, Thomas B., Johns Hopkins University.
Harnly, Morris H., Student, Columbia University.
Hartwell, Rhoda A., Assistant, Illinois University.
Helwig, Edwin R., Student, University of Pennsylvania.
Herdman, Emma C., Milet Road, Liverpool, England.
Hopkins, Aubrey E., Graduate Student, Harvard University.
Hoy, William E., Jr., Professor of Biology, Presbyterian College of South Carolina.
Kiernan, Grace R., Instructor of Biology, Miami University.
Knapke, Bede, Teacher, St. Bernard College.
Lewis, Ruth W., Investigator, Harvard University.
Lucas, Alfred M., Wilton, Connecticut.
MacDougall, Mary S., Head of the Biological Department, Agnes Scott College.
Moses, Mildred S., Research Assistant, Carnegie Institution of Washington.
Pennypacker, Miriam I., University of Pennsylvania.
Plunkett, Charles R., Columbia University.
Ratcliffe, Francis N., Student, Oxford University.
Rice, Kenneth S., Assistant Professor of Biology, Clark University.
Sandison, James C., Assistant in Anatomy, University of Georgia.
Scott, Miriam J., Student, University of Pennsylvania.
Stewart, Dorothy R., Bryn Mawr College.
Strauss, Maurice B., Amherst College.
Sturtevant, Phoebe R., Carnegie Institution.

Tilden, Evelyn B., Technician, Rockefeller Institute.
Thillayampalam, E. M., Graduate Student, Columbia University.
Valentine, J. Manson, Assistant, Yale University.
Varian, Basil B., Assistant, University of Pennsylvania.
Vicari, Emilia M., Research Scholar, Columbia University.
Visscher, Maurice B., Assistant, University of Minnesota.
Wallace, Edith M., Carnegie Institution.
Willis, Josephine, University of Pennsylvania.

PHYSIOLOGY—Independent Investigators.

Adolph, Edward F., Instructor in General Physiology, University of Pittsburgh.
Amberson, William R., Assistant Professor, University of Pennsylvania.
Bailey, Percival, Director, Laboratory of Surgical Research, Harvard University Medical School.
Bazett, Henry C., Professor of Physiology, University of Pennsylvania.
Bradley, Harold C., Professor of Physiological Chemistry, University of Wisconsin.
Bronfenbrenner, J. J., Associate Member in Pathology and Bacteriology, Rockefeller Institute.
Chown, Bruce, Assistant Dispensary Physician, Johns Hopkins Hospital.
Clark, Jefferson H., Instructor in Pathology, Medical School, Temple University.
Clowes, G. H. A., Director of Research, Eli Lilly & Co.
Cohn, Edwin J., Assistant Professor of Physical Chemistry, Harvard University Medical School.
Edwards, Dayton J., Associate Professor, Cornell University Medical College.
Garrey, Walter E., Professor of Physiology, Tulane University.
Harvey, Edmund Newton, Professor of Physiology, Princeton University.
Hecht, Selig, Research Fellow, Harvard University.
Hinrichs, Marie A., Laboratory Assistant, Nela Research Laboratory.
Holt, L. Emmett, Jr., Instructor, Johns Hopkins University.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Professor of General Physiology, The University of Chicago.
Loeb, Leo, Professor of Pathology, Washington University Medical School.
Lyon, E. P., Professor of Physiology, University of Minnesota.
Mathews, A. P., Professor of Biochemistry, University of Cincinnati.
Pond, Samuel E., Instructor in Physiology, Washington University.
Querido, Arie, Harvard University Medical School.
Redfield, A. C., Assistant Professor of Physiology, Harvard University Medical School.
Robertson, William E., Professor of Medicine, Medical School, Temple University
Schwitalla, Alphonse, Associate Professor of Biology, St. Louis University.
Shapley, Harlow, Director, Harvard Observatory, Cambridge, Mass.
Smith, Homer W., Harvard University Medical School.
Spaeth, Reynold A., Visiting Professor of Physiology, Rockefeller Foundation.
Warren, H. C., Professor of Psychology, Princeton University.
Woodward, Alvalyn E., Associate Professor, North Carolina College for Women.
Wulzen, Rosalind, Instructor, University of California.

PHYSIOLOGY—Beginning Investigators.

Baker, Herbert N., Johns Hopkins University.
Brown, D. E. S., University of Michigan.
Cattell, Ware, Garrison, New York.
Egloff, William C., University of Chicago.
Genther, Ida T., Student, Mount Holyoke College.
Glusker, S. David, University of Pennsylvania.
Hartline, H. K., Lafayette College.
Hurd, A. L., Harvard University Medical School.
Kredel, Frederick E., Graduate Assistant in Zoölogy, University of Pittsburgh.
Landis, Eugene M., Student, University of Pennsylvania.
Nadler, Jacob E., Assistant in Biology, Johns Hopkins University.
Page, Irvine H., Research, Eli Lilly & Co.
Studebaker, Mabel T., Chemist, Eli Lilly & Co., Indianapolis.
Thomas, Giles W., Research Assistant, Harvard University Medical School.
Walden, Eda B., Research Chemist, Eli Lilly & Co.
Walden, Geo. B., Research Chemist, Eli Lilly & Co.

BOTANY—Independent Investigators.

Allen, Charles E., Professor of Botany, University of Wisconsin.
Brooks, Matilda M., Hygienic Laboratory, United States Public Health Service, Washington, D. C.
Brooks, Sumner C., Hygienic Laboratory, United States Public Health Service, Washington, D. C.
Cleland, Ralph E., Associate Professor of Biology, Goucher College.
Cowdry, Nathaniel H., 412 Hawthorn Road, Roland Park, Baltimore, Md.
Gates, Reginald R., Professor of Botany, University of London, Kings College.
Hazen, Tracy E., Assistant Professor of Botany, Barnard College, Columbia University.
Inman, Ondess L., Professor of Biology, Antioch College.
Lewis, Ivey F., Professor of Biology, University of Virginia.
Schramm, J. R., Professor of Botany, Cornell University.
Stevens, Neil E., Pathologist, Department of Agriculture, Washington, D. C.
Stokey, Alma G., Professor of Botany, Mount Holyoke College.
Taylor, William Randolph, Assistant Professor, University of Pennsylvania.
Wann, Frank Burkett, National Research Fellow, Cornell University.

BOTANY—Beginning Investigators.

Emerson, Robert, Harvard University.
Fogg, John Milton, Jr., University of Pennsylvania.
Hof, Anne, Watertown Arsenal, Watertown, Massachusetts.
Holden, Alan Nordby, Harvard University.
Keefe, Anselm M., Fellow in Botany, University of Wisconsin.
Kemp, Margaret, Investigator, Radcliffe College.
Wilcox, Marguerite S., Laboratory Assistant, Department of Agriculture, Washington, D. C.

STUDENTS.

1924.

Botany.

Beall, Ruth, Assistant in Biology, Goucher College.
Bell, Hugh Philip, Associate Professor of Biology, Dalhousie University.
Betts, Edwin M., Student, University of Virginia.
Boughton, Esther Marie, Graduate student, Elmira College.
Bunten, Eva Isabel, George Washington University.
Canby, Margaret Leslie, Student, Pomona College.
Degener, Otto, Amherst, Mass.
Flexner, William Welch, Student, Harvard College.
Fuller, Annie Mae, Louisiana State University.
Hart, Helen T., Assistant in Botany, Vassar College.
Koch, Ninna F., Graduate student, Tulane University.
Levy, Celia D., Hunter College.
Neal, Mary Eulalia, Teacher of Science, Boston Schools.
Nicholson, James Lawrence, Student, Oberlin College.
Poole, James P., Assistant Professor of Evolution, Dartmouth College.
Sabine, Janet, Student, Radcliffe College.
Schimian, Gervase, High School Instructor, St. Vincent College.
Stuart, Dorothy Rhett, Graduate student, Johns Hopkins University.
Showers, Edith, Smith College.
Walsh, Lydia Bourne, Wellesley College.

Embryology.

Allen, Louise Rector, Instructor, Vanderbilt University.
Brown, Alice E., 924 Newington Ave., Baltimore, Md.
Chase, Elton Fletcher, Instructor, Tufts College.
Clapp, Eunice E., Student, Smith College
Curwen, Alice Osborne, Student, Smith College.
Diller, William F., Jr., Instructor, University of Pennsylvania.
Dowling, Alexander Scott, Student, DePauw University.
Fales, Doris E., Student, Mount Holyoke College.
Giang, Frederica Hsien Tren, Student, Vassar College.
Leonard, Donald William, Wesleyan University.
Mead, Harold Tupper, Associate Professor Zoölogy, Tulane University.
Meader, Ralph G., Student Assistant in Zoölogy, Ohio Wesleyan University.
Miller, Ralph English, Assistant Instructor, Dartmouth College.
Mullin, Catharine, Instructor in Zoölogy, State University of Iowa.
Orr, Paul Rudbert, University of Pennsylvania.
Parpart, Arthur K., Student, Amherst College.
Rankin, Iva Lucille, Assistant Instructor, University of Wisconsin.
Rowland, Virginia, Mount Holyoke College.
Roxas, Hilario A., Student, University of Chicago.
Sanborn, Irene Hannah, Student, Simmons College.
Sanders, Elizabeth Percy, Assistant in Biology, Goucher College.
Scott, J. Allen, Instructor, University of Vermont.

Sleeter, Victor R., Asst. in Embryology Laboratory, Illinois Wesleyan University.

Spencer, Warren Poppino, Ohio State University.

Stein, Kathryn F., Mount Holyoke College.

Urbantke, Elsie, Instructor in Histology and Embryology, University of Texas, Medical branch.

Wenstrup, Edward Joseph, Teacher of Zoölogy, St. Vincent College.

Williams, Elisabeth, Mount Holyoke College.

Winkley, Ruth, University of Michigan.

Physiology.

Atlee, J. L., Jr., Lancaster, Pennsylvania.

Castle, Edward Sears, Harvard University.

Clarke, Miriam F., Assistant in Physiological Laboratory, Mount Holyoke College.

Gray, Philip Lewis, Instructor in Biology, Hamilton College.

Hanger, Irwin C., Staunton, Virginia.

Haywood, Charlotte, 11 Haewood St., Lynn, Mass.

Kenyon, Walter A., Instructor in Biology, Hamline University.

Merritt, Olive Eleanor, Instructor in Biology, Hunter College.

Miles, Alice L., Laboratory assistant, Yale University.

Millemann, Raymond Joseph, Student, Cornell Medical College.

Pereira, Jayme Regallo, Assistant in Clinical Medicine, Rio de Janeiro, Brasil.

Reed, Lucille Lyman, Sophie Newcomb College.

Root, Walter Stanton, Assistant in Biology, Wesleyan University.

Tychowski, Dr. Wiktor, Zbigniew, Poland, Europe.

Wayman, Marguerite, Assistant Professor, Hunter College.

Williams, S. Culver, Assistant in Biology, Yale University.

Wright, Sewall, Senior Animal Husbandman, Washington, D. C.

Wright, Sydney L., Jr., Chemist, Pennsylvania Hospital.

Protozoölogy.

Cass, Mildred Frances, Minor Assistant, Columbia, University.

Collier, Jane, Technician, Harvard Medical School.

Eickmann, Reintraut W. M., Graduate Student, Columbia University.

Fogg, Lloyd Clarke, Graduate Student, Dartmouth College.

Greiner, Bright E., Instructor Zoölogy, Syracuse University.

Hole, Frances L., Instructor, Doane College.

Halsey, Harvey Randolph, Laboratory Instructor, Columbia University.

Keith, Wilma, High School teacher of Biology, 1229 Carr Ave. Memphis, Tenn.

Li, Ju Chi, Student, Columbia University.

Little, Malcolm Edgeworth, Laboratory Assistant, Columbia University.

Messer, Harold Madison, Instructor in Biology, Washington Square College, N. Y. University.

Oakes, Mervin E., Instructor, Washington Sq. College, N. Y. University.

Philpott, Charles H., Professor of Biology, University of Missouri.

Pickwell, Gayle B., Instructor in Zoölogy, Northwestern University.

Pirkle, Ruth Janette, Instructor in Biology, Agnes Scott College.

Schultz, Jack, Columbia University.

Taft, Charles H., Jr., Assistant Professor, Tufts College.

Attending Morning Lectures Only.

Bertsch, Marguerite, Columbia University.

Zoölogy.

Abrams, Eleanor L., Mount Holyoke College.
Albro, Helen Tucker, Instructor in Zoölogy, Mount Holyoke College.
Appel, Fred W., Graduate Student, University of Chicago.
Birrell, Ethel Jane, Science Instructor in State Normal School, Danbury, Conn.
Bradner, John, Student, Yale University.
Bray, C. Russell, DePauw University.
Brenner, Nancy Jane, Hunter College.
Brode, Malcolm D., Assistant, University of Chicago.
Brummer, Freda Louise, Student, University of Pennsylvania.
Canby, Caroline Prescott, Student, Radcliffe College.
Carr, Ralph William, Wesleyan University.
Coolman, Raymond, Student, Wabash College.
Cummings, Elizabeth Frances, Student, Radcliffe College.
Curry, Laliah Florence, Wellesley College.
Dobroscky, Irene Dorothy, Student, Cornell University.
Forer, Robert, Student, Rutgers College.
Fowler, Katharine Stevens, Bryn Mawr College.
Goldman, Douglas, Student, University of Cincinnati.
Haas, Edna L., Connecticut College for Women.
Hall, Harris Tremaine, Student, Princeton University.
Harris, Dorothy Lou, Wilson College.
Hayden, Catherine Spencer, Goucher College.
Heys, Florence M., Assistant in Zoölogy, Washington University.
Hunter, George William, Jr., Illinois University.
Jaleski, Clarence Thomas, Butler College.
Kottmeier, Marjorie, Knox College.
Kropp, Benjamin, Graduate Student, Harvard University.
Leich, Charles F., Evansville, Indiana.
Lynch, James E., Assistant in Anatomy, Washington University.
MacDonell, Thomas Kennerly, Graduate Fellow, Emory University.
McQuilkin, William Everett, Student, Doane College.
Miller, David K., Illinois Wesleyan University.
Northrup, Flora E., Graduate Assistant, Washington University.
Parmelee, Eleanore W., Graduate Fellow, Mt. Holyoke College.
Pike, Radcliffe Barnes, Bowdoin College.
Quick, Mary Elaine, Geo. Washington University.
Raffel, Daniel, Student, Johns Hopkins University.
Roberts, Joseph Marvin., Laboratory Assistant in Zoölogy, Illinois Wesleyan University.
Russell, Mary Anna, Goucher College.
Sayle, Mary Honora, Assistant Instructor, University of Wisconsin.
Schulze, Lula May, Assistant in Zoölogy, University of Missouri.
Stephens, Mary Allen, Elmira College.
Swan, Dorothy M., Colorado College.
Sweeting, Marjorie A., Barnard College, Columbia University.

TeWinkel, Lois E., Oberlin College.
Tyson, John Joyner, Ayden, North Carolina.
Warren, A. Emerson, Student, and Assistant, Yale University.
Wenner, William Franklin, Assistant Instructor, Yale University.
Williams, Elizabeth T., Smith College.
Zeman, Mira, New York University.

3. TABULAR VIEW OF ATTENDANCE.

	1920	1921	1922	1923	1924
Investigators—Total	136	172	182	176	194
Independent:					
Zoölogy	69	79	87	90	77
Physiology	22	26	28	23	33
Botany	7	13	15	13	14
Under Instruction:					
Zoölogy	29	34	34	41	47
Physiology	7	11	11	5	16
Botany	2	9	7	4	7
Students—Total	120	120	126	146	134
Zoölogy	56	53	59	59	50
Protozoölogy	15	11	12	16	17
Embryology	26	28	28	31	29
Physiology	15	18	19	22	18
Botany	8	10	8	18	20
Total Attendance	256	292	308	322	328
Institutions Represented—Total	86	95	104	107	110
By investigators	55	67	71	62	69
By students	57	53	68	73	68
Schools and Academies Represented					
By investigators	1	–	–	–	–
By students	7	–	–	4	–

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1924.

Amherst College
Antioch College
Barnard College
Bowdoin College
Bryn Mawr College
Butler College
Carnegie Institution, Cold Spring Harbor
Carnegie Institution of Washington
Clark University
Columbia University
Cornell University
Cornell University Medical College
Dartmouth College
DePauw University
Doane College
Elmira College
George Washington University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Illinois Wesleyan University
Johns Hopkins University
Knox College
Eli Lilly & Co.
Massachusetts Institute of Technology
McGill University
Miami University
Mount Holyoke College
Nela Research Laboratories
New York University
North Carolina College for Women
Northwestern University
Oberlin College
Ohio Wesleyan University
Presbyterian College of South Carolina
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers College
Simmons College
Smith College
Sophie Newcomb College
St. Bernard College
St. Louis University
St. Norbert's College
St. Vincent College
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Philippines
University of Virginia
University of Vermont
University of Wisconsin
U. S. Dept. of Agriculture
Vanderbilt University
Vassar College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Wilson College
Wistar Institute of Anatomy and Biology.
Yale University

SCHOLARSHIP TABLES

The Lucretia Crocker Scholarships for Teachers in Boston, Since 1888.

Scholarship of $100.00, Supported by a Friend of the Laboratory, Since 1898.

The Edwin S. Linton Memorial Scholarship of Washington and Jefferson College.

5. EVENING LECTURES, 1924.

Thursday, July 3,	Dr. W. J. Crozier	"The Nature of a Central Nervous Process."
Friday, July 11,	Dr. E. J. Cohn	"The Physico-chemical characterization of Proteins."
Tuesday, July 15,	Dr. Merkel H. Jacobs	"Some introcellular changes dependent on differential cell permeability."
Friday, July 18,	Dr. L. L. Woodruff	"The Physiological Significance of Conjugation and Endomixis."
Tuesday, July 22,	Dr. W. B. Cannon	"Some Revelations of the Denervated Heart."
Friday, July 25,	Dr. C. R. Moore	"The Temperature Regulation of the Mammalian Testis and its Relation to Viability in Grafts and Displacements."
Tuesday, July 29,	Dr. Ralph S. Lillie	"Structure and Function in Protoplasmic Systems with Special Reference to the Conditions of Transmission and Recovery."
Friday, August 1,	Dr. C. E. Allen	"Studies of Inheritance in Sphaerocarpus."
Tuesday, August 5,	Dr. J. K. Breitenbecker	"Genetics of Bruchus."

Tuesday, August 26,

Dr. Julian Huxley............"Bird Courtship and the Problem of Sexual Selection."

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6. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G, Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.
Marrs, Mrs. Laura Norcross, 9 Commonwealth Ave., Boston, Mass.
Mason, Miss E. F., 1 Walnut St., Boston, Mass.
Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.

MINNS, MR. THOMAS, 14 Louisburg Square, Boston, Mass.
MORGAN, MR. J. PIERPONT, JR., Wall and Broad Sts., New York City, N. Y.
MORGAN, PROF. T. H., Columbia University, New York City, N. Y.
MORGAN, MRS. T. H., New York City, N. Y.
NOYES, MISS EVA J.
NUNN, MR. LUCIAN L., Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York City, N. Y.
PHILLIPS, DR. JOHN C., Windy Knob, Wenham, Mass.
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EGG-VOLUME AND FERTILIZATION MEMBRANE.

CHARLES D. SNYDER.

The question of fertilization and egg-volume seems to come up periodically for renewed research and discussion in spite of the papers already written on the topic.[1] In view of the disagreement as to the facts of volume changes at the moment of fertilization the writer wishes to submit the following evidence that was put in a paper in December, 1904, from observations made in that year on two species of echinoderms, and that hitherto has not been published. The work was done at the Timothy Hopkins Sea-side Laboratory of Stanford University, California.

The microscope images of the eggs were projected by a camera lucida, and outline drawings were made of their greatest diameters. Upon each sheet of drawings a stage micrometer scale was also projected and drawn. The magnification was uniform throughout. The diameters given in sections 1–4 inclusive, of this paper, are of these drawings, and should be divided by 110 in each case to get the approximate natural diameter.

1. *Echinarachnius eccentrica.*[2] Eggs made to develop parthenogenetically by treatment with hypertonic salt solution are known to shrink and then to swell again upon return to normal sea-water. Mature eggs of *Echinarachnius eccentrica* put in optimum hypertonic solution for 1½ hours were observed to shrink from a mean normal diameter of 13.27 to a mean diameter of 11.6 mm. After return to normal sea-water for 1 hour the eggs showed diameters ranging from 12.86 to 13.18 mm. By the time cleavage began, and the eggs were still in 1 and 2-cell stages, the diameters ranged between 14 and 16 mm.; when in stages varying from the 4-cell to the morula stage, the diameters ranged between 14.5 and 18 mm. These measurements refer to the egg-cytoplasm, no membranes appearing on eggs so treated.

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[1] For review of the literature and discussion of the problem see Lillie, F. R., "Problems of Fertilization," Chicago, 1919, pp. 147–154; also Glaser, Otto: "Fertilization, Cortex and Volume," Biol. Bull., Vol. XLVII., pp. 274–283, 1924.

[2] Thanks are due to Professor Harold Heath, who kindly identified this species of sand-dollar for the writer.

Of the eggs fertilized with sperm those in 1 to 4-cell stages had diameters of egg-cytoplasm ranging from 13 to 15, and of fertilization membrane from 14.7 to 18 mm.; those in stages from 8-cells to blastulæ had diameters from 14.5 to 16 mm. for egg-cytoplasm, and 16.5 to 17 mm. for fertilization membrane. The number of eggs measured in each case was five only. But the extremes of a large number as well as mean sizes were taken in each case. The mean size of the hypertonic parthenogenetic eggs thus appear to reach nearly the same limit that the membrane does in eggs fertilized with sperm; the egg-cytoplasm of the former apparently takes up water as easily as the membrane of the latter. The writer's observations did not include a large enough number of these eggs at the moment of membrane formation to determine whether or not they showed a preliminary shrinking, as Otto Glaser[1] and others have observed in eggs of other species. One can only say that if the shrinking took place it must have occurred between the moment of the entrance of the spermatozoön and the moment just before the first cleavage.

2. *Asterina miniata* (*Brandt*) *Perrier*.[3]—When mature, and just before fertilization, a sample of seven eggs of this species measured 19, 19, 18.5, 19, 20, 19 and 20 mm., giving a mean of 19.25 mm. and an average of 19.21 mm. in diameter. When treated with suitable amounts of citric acid for a while and then returned to normal sea-water the eggs began to go into cleavage and develop into blastulæ.[4] A fertilization membrane could be seen reaching across the furrows between the cells of these eggs. They differed in this respect from the sand-dollar eggs that were made to go into cleavage by hypertonic solutions. But nevertheless the membranes did not free themselves from the outermost periphery of the egg-cytoplasm. The diameters, therefore, of egg-cytoplasm and fertilization membrane of *Asterina miniata* so treated underwent equal changes; they ranged between 19 and 21, giving a mean diameter of 20 mm. No measurements were made at the moment of treatment with the acid.

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[3] Thanks are due to Dr. W. K. Fisher, who kindly identified this species of Asterina for the writer.

[4] The eggs of this species are known to be somewhat naturally parthenogenetic. In the series with the optimum concentration of the citric acid 79 per cent. swimming larvæ appeared at the end of 42 hours whereas in the control only 6 per cent. of the eggs were segmented and none were swimming. The average of all the series worked with showed a natural parthenogenesis to the extent of about 3.5 per cent.

When mature eggs of *Asterina miniata* are treated with sperm, the egg-cytoplasm during early stages of cleavage has an average diameter of 17.7 and the fertilization membrane one of 21 mm. This is shown in the table.

EGGS OF *Asterina Miniata* FERTILIZED WITH NORMAL SPERM.

Stage of Cleavage.	Diam. of Egg Cytoplasm.	Diam. of Fertilization Membrane.	Stage of Cleavage.	Diam. of Egg Cytoplasm.	Diam. of Fertilization Membrane.
1-cell.....	18	20	2-cell.......	18	20
1-cell.....	17	20.5	2-cell.......	18.5	22
1-cell.....	17	20	2-cell.......	18.7	21
1-cell.....	17	21.5	2-cell.......	17.5	22
1-cell.....	18	22.5	2-cell.......	18.5	22
1-cell.....	18	25	2-cell.......	18.7	20.5
4-cell.....	18	21	2-cell.......	19.2	22
2-cell.....	15	20	2-cell.......	19.0	20.5
2-cell.....	15	20	Average....	17.7	21.0

The mean diameters in these observations are 17.1 for the egg-cytoplasm and 22.5 for the membrane. The averages thus show less deviation than do the mean numbers. The amount of shrinking of the egg-cytoplasm comparing the mean diameters before and after fertilization is 11.1 per cent.; comparing the average diameters it is 7.8 per cent.

During the early stage of normal fertilization, then, the eggs of *Asterina miniata* may be said to show a marked shrinking of the egg-cytoplasm.

These figures are of the same order as Glaser (1914) observed for the reduction in diameters of just fertilized *Arbacia* eggs (from 1.4 to 14.5 per cent.) and *Asterias* eggs (from 10 to 17 per cent.); and Okkelberg [5] in volume reduction of eggs of the brook lamprey, 13.4 per cent. Just lately (1924) Glaser [1] repeated the measurements of Arbacia, using an improved method in order to prevent possible flattening of eggs before the membrane has been elevated (first suggested by Reighard, see Okkelberg, *loc. cit.*, p. 97, footnote 2), and finds that the percentage reduction of diameter is less than in his earlier work, but still a demonstrable mean of 3 per cent.

4. In spite of the meagerness of observations (the lack of measurements on the sand-dollar egg during the earliest stages following insemination, the lack of a more perfected treatment to

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[5] Okkelberg, "Volumetric Changes in the Egg of the Brook Lamprey . . . after Fertilization," BIOL. BULL., Vol. XXVI., pp. 92–99, 1914.

induce parthenogenesis) the data still furnish one or two points of further interest.

The shrinking of the eggs of *Echinarachnius eccentrica* when subjected to optimum hypertonic solution for parthenogenetic development was from the mean diameter 13.27 before, to one of 11.6 mm. at the end of the treatment, or a reduction of 12.6 per cent. After return to normal sea-water, and by the time cleavage completed the 2-cell stage, the mean diameter was 15 mm.—an increase of 13 per cent.; by the time the eggs were in stages ranging from 4-cell to morulæ the mean diameter was 16.2 mm. showing a total increase of 22 per cent.

In the case of the inseminated eggs of this species the fertilization membrane (assuming it to be present on the unfertilized egg) showed an increase, while developing to the 4-cell stage, from a mean of 13.27 to one of 16.3 mm.—an increase of 22.8 per cent.; eggs in stages of 8-cells to blastulæ showed an increase to a mean diameter of 16.75 mm. or a mean total increase of 26.2 per cent.

In the case of *Asterina miniata* the increase in the average total diameter of fertilization membrane of inseminated eggs is from 19.21 to 21.0 scale divisions or one of 9.3 per cent, comparing averages the increase is 16.8 per cent.; of the acid treated parthenogenetic eggs the increase of both egg-cytoplasm and fertilization membrane (as in the case of hypertonic parthenogenetic eggs of the sand-dollar) is equal, and is the difference between 19.25 and 20.0 mm., or an increase of 3.9 per cent.

5. It may be of further interest to calculate the approximate mean actual diameters and actual volumes[6] of these eggs. If we assume them to have been spheres in all cases we have the following: The mature unfertilized egg of *Echinarachnius eccentrica* has a mean diameter of 120 μ, from which its volume must be about .0009 mm.^{3}; the optimum hypertonic shrinking gave a diameter of 105 μ, or a volume of .00061 mm.^{3}, representing a volume reduction of 33 per cent.

The mean actual diameter of mature *Asterina miniata* eggs is 174.6 μ, representing a volume of .00278 mm.^{3}; the mean diameter of egg-cytoplasm just after fertilization is about 161 μ, from which the volume must be about .00217 mm.^{3}, representing a volume reduction of 21.9 per cent. The fertilization membrane on the other hand presented a diameter of 191 μ and therefore enclosed a volume of .00364 mm.^{3}; this represents an increase of volume capacity of 30.8 per cent.

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[6] These volumes are calculated by multiplying the cube of the radius by 4.18 in each case. The radius is found by dividing the mean projected greatest diameter of the egg (that given in the text) by 2 x 110, 110 being the magnification.

The above observations are summed up in the following syllabus for the purpose of ready comparison. It will be observed that the ratio of diameter and volume changes is roughly as 1 : 2.8, a purely geometrical ratio.

SUMMARY.

Percentage Dimensional Changes in Two Species of Echinoderm Eggs.

Reduction in egg-cytoplasm:
- Of *Echinarachnius eccentrica*
  - By hypertonic salt action—in diameter, 12.6; in volume, 33.3.
  - By insemination (not observed).
- Of *Asterina miniata*
  - By citric acid treatment (not observed).
  - By insemination—in diameter, 7.8; in volume, 22.

Swelling of fertilization membrane:
- Of *Echinarachnius eccentrica*
  - Parthenogenetic (hypertonic salt action)[7]—in diameter, 22.8; in volume, about 62.
  - Normally inseminated—in diameter 26; in volume, about 70.
- Of *Asterina miniata*
  - Parthenogenetic (citric acid treatment)[4]—in diameter 3.9; in volume, about 11.
  - Normally inseminated—in diameter 9.3; in volume, 31.

No special technical procedure was resorted to, to make certain that the eggs in these experiments were always spherical. The writer cannot, therefore, be quite certain that the diameters given above are those of perfect spheres and accordingly that the volumes given are the exact volumes of the eggs observed. If, as has been pointed out by others, the eggs at one time may be disks flattened vertically (Reighard, Chambers [8]) and at another time ellipsoids, or pear-shaped objects, suspended at one end of their long axes, and yet at another time perfect spheres, then there is an unrelated error among the observations that renders them worthless.

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[7] The membrane is assumed to be present here and coextensive with the egg-cytoplasm.

In the cases here studied there exists only the possibility of the eggs being flattened vertically during the period before fertilization and then changing into spheres after the fertilization membranes are raised from the egg-cortex. This, however, affects only the reduction observed in the inseminated *Asterina* eggs.

No one will doubt that the observed reduction in size of the sand-dollar eggs during the bath in hypertonic salt represents a real reduction in volume. If the effect of the osmosis is a gelation there may have been a hardening of the egg-cytoplasm, but this hardening of itself could not change the egg-mass from a spheroid to a sphere. If the effect of the osmosis is an increase in surface tension then such a change in form may well take place.

The reduction in diameter of the inseminated *Asterina* eggs during the first stage of cleavage is of the same order of magnitude as was observed in the sand-dollar eggs in their hypertonic bath. While some of this reduction may have been due to reshaping, there also can be no doubt that some of it was due to loss of material on the part of the egg-cytoplasm. This material, as others have maintained, may be colloidal in part, but this observation supports the view that considerable water is given off from the egg together with the colloid.

It is remarkable that the percentage of swelling of the egg treated to the hypertonic salt bath, after return to normal sea-water, and the percentage swelling of the fertilization membrane of the inseminated egg should both be of the same order of magnitude. This swelling in neither case can be due to any considerable extent to a reshaping of egg-substance. However evident it is that the egg-cytoplasm of this parthenogenetic egg has undergone a change in permeability different from the egg-cytoplasm of the normally inseminated egg, it nevertheless appears that the limit to extension in the one case is the same as it is in the other. For since the beautiful demonstration of Chambers [8] there can be no doubt that the fertilization membrane is a preëxistant structure. One may take the limit in the swelling, therefore, to be the degree of permeability and elasticity of the membranes in both cases.

What has just been said of the eggs subjected to hypertonic solution appears also to be true of the eggs subjected to citric acid. For in this latter case the egg-cytoplasm also swells coextensively with the egg-membrane. It will be remembered that in these eggs the membrane could be seen bridging the furrows between the cells after cleavage, in this respect differing from the hyaline membrane that is observed to dip down and follow the furrow closely.[8] The limit to swelling in the artificially parthenogenetic Asterina egg thus also lies in the egg (fertilization) membrane. Only, the citric acid treatment seems to render the membrane somewhat less permeable to water or less elastic, or both less permeable and less elastic, than does the treatment with normal sperm.

In conclusion it may be added that while the above observations contribute little or nothing of a decisive character to the problem, they nevertheless do add to the attractiveness of the space-time method. With the newer technique a thoroughly systematic application ought to yield results not only decisive but also important.

The Johns Hopkins University,
December 18, 1924.

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[8] Chambers, Biol. Bull., Vol. XLI., pp. 318–350, 1921.

THE EFFECTS OF DISLOCATION OF THE EYE UPON THE ORIENTATION AND EQUILIBRIUM OF THE GOLDFISH (*CARASSIUS AURATUS*).

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(From the Hull Physiological Laboratory of the University of Chicago).

It has long been known that the eyeballs of various fishes show different movements during locomotion. It is also established that the body movements have an influence on the movements of the eyes.

Lyon [1] has shown that the dogfish compensates rotation about a dorsoventral axis by moving its eyes in the opposite direction. "The eyes," states Lyon, "sometimes show the same motions when the animal moves voluntarily and normally." "A dogfish, for example, when swimming on its side may keep the upper eye to the ventral, the lower one to the dorsal side of the orbit. Compensatory motions are not, therefore, confined to passive rotation by external means."

Lyon found that these compensatory motions of the eyeballs in fish are practically independent of the sense of light, for they cannot be abolished by blinding (transection of the optic nerves). He also found a causal relationship between the semicircular canals and these eye motions.

The opposite question, whether the eye or visual impressions can influence the body orientation and movements in fish has also been investigated but only in reference to the positive and negative heliotropism. Parker [2] has shown that in an unilluminated field dogfish will swim toward a single light, *i.e.*, they are positively phototropic. Thus the light has a stimulating effect on the progressive movements of the fish and Parker concludes that the retinal image is an important factor in guiding the locomotion of these fishes.

Admitting the fact of positive phototaxis in fishes, there still remains the possibility that in diffused light visual impulses or the fields of vision as a whole may influence the orientation, despite the fact as stated by Lee [3] that "the blinded fish swims normally in all respects."

Numerous experiments by Loeb, Bethe, Bigelow, Lee, Parker, Maxwell, etc., have shown that the labyrinths are concerned with the equilibrium of the fish. The orientation of fish deprived of their labyrinths is very faulty or erratic.

Comparing the striking effects on orientation of labyrinth extirpation with the absence of effects from blinding appears to indicate that the retinal image is not an important factor in equilibrium and orientation in diffused light.

The usual method of studying the influence of the eye on orientation has been that of removal. The difference in the animal's behavior before and after blinding has been interpreted as being due to the removal of the visual function and therefore it is to be considered as representing the normal effect of vision upon orientation and locomotion.

We attempted to approach the problem from a different angle. We tried to investigate the rôle of vision in orientation not by removal but by *dislocation* of the eye, hoping that while the dislocated eye would still perform its visual function, the animal's equilibrium and orientation could be observed and the problem, whether or not the eyes influence orientation, put to a crucial test.

EXPERIMENTAL.

Large goldfish (*Carassius auratus*), eight and twelve inches in length, were chosen for the work because of their availability and especial suitability.[1]

The skull of the goldfish is high, forming a large intracranial space over the brain which permitted the operative work to be done readily. The method chosen was that of dislocation of *one* eye and removing the other. Dislocation was performed by inserting the eye with its nerve and blood supply intact into an artificial orbit placed higher in the skull. This was accomplished in the following way:

A hole about the size of the eye was drilled in the top of the head at a point in the vertical plane of the eyes just to the left of the midsaggital line. In the skull and underlying tissue between the left orbit and the drilled hole a narrow channel was cut (including the orbital wall) and thus a communication between the natural and artificial orbit produced. The eyeball was slid along the prepared channel and into the drilled hole, which then served as an artificial orbit. This dislocation can be done very easily and without any force. Neither the eye muscles, nor the nerves of the dislocated eyeball were cut or obviously injured and even the major part of the conjunctiva bulbi and sclerae can be left intact.

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[1] We acknowledge with pleasure our thanks to Mr. Parker and Mr. Young of the Lincoln Park Zoölogical Gardens, who kindly supplied us with the fish used in this experiment.

The artificial orbit is able to hold the dislocated eye in place indefinitely. At the replacement the dislocated eye showed some healing in the new orbit.

The experiment was successful on two animals. In the third fish a fungus infection on the dislocated eye caused death five days after the operation. To prevent such infections a daily potassium-permanganate (weak solution) bath of the entire fish is necessary.

Immediately following the operation (the first one performed on May 15, 1924) no abnormalities in the animal's behavior were observed. After keeping them under observation for a few days the other eye was removed (May 20, 1924). We were able thus experimentally to produce real cyclopy. These cyclops in their first week behaved exactly like normal blind fish, they swam and oriented themselves quite as before operation. However, after about the tenth day (in our first protocol: June 1, 1924), the animal was observed to tilt the body somewhat to the left. When at rest it assumed a position with its dorsoventral axis several degrees to the left of the vertical. This position was maintained during swimming.

The tilting of the body toward the side in which the eye was dislocated increased day by day, reaching its maximum in about four weeks (in the case of our first animal: June 27, 1924). This maximum tilting was about 45° and was permanent so long as the eye remained in its new orbit.

The gross anatomy of the dislocated eye was quite normal. The media were clear. We observed also some *oscillatory movements* of the dislocated eyes, when we took the fish out of water.

The vision of the fish with dislocated eyes was tested during the period of the last three weeks of the experiment and found to be very good. If a small rod was slowly moved toward the eye the animal quickly turned aside and avoided it constantly.[1] The animal showed in all respects the behavior of a fish in possession of its visual function it avoided all kinds of obstacles. The fishes did not show the phenomenon which Parker described concerning blind fish, *i.e.*, they remain usually near the bottom and swim about in such a way as to be almost continually in contact with some solid surface, as though relying on its sense of touch for its location.

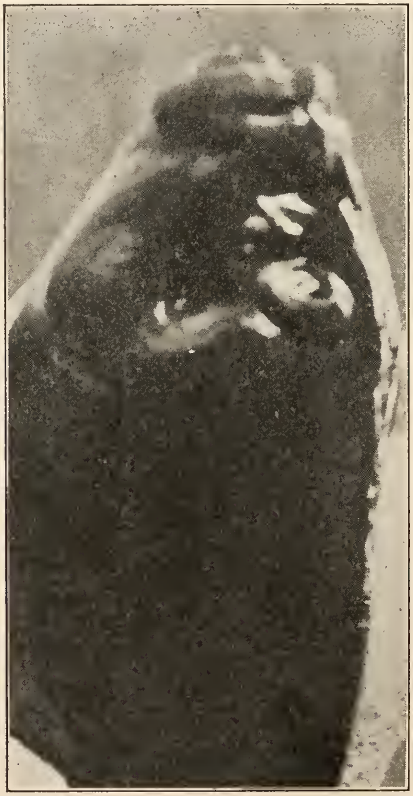

FIG. 1. Goldfish, with the dislocated eye. The other eye removed.

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[1] When the animal was near the surface it avoided the rod before it reached the water.

On the 27th of June the dislocated eye was removed from the artificial orbit and slid again along the channel into the original orbit. This operation is also a very easy procedure and can be done without obvious injury of the eye or of the animal.

The animal, as most Anamnia, recovered very slowly from anesthesia and therefore we began our observations on the following day (June 28). The fish was in the normal position at rest and during locomotion. In the several weeks following, the orientation and locomotion were constantly normal, no tilted positions were observed. The other goldfish showed also two weeks after the operation the above described tilting and the tilting reached its maximum six weeks after the dislocation. The results obtained in our second experiment corroborate completely the observations on our first fish with dislocated eye, since in the second animal tilting and the return of vision occurred also synchronously.

At no time during the experiment did the animal show any abnormalities other than the tilting. There were never any evidences of circus movements, etc., which may follow injuries of the midbrain, medulla and labyrinth (Steiner,[4] Loeb,[5] Bethe,[6] Bigelow,[7]).

Discussion.

"A normal fish has a delicate sense of the distance involved in swimming in a straight line. This is shown by the remarkable skill with which he avoids obstacles; in swimming around his aquarium constantly, he strikes his nose directly against the side of the tank comparatively rarely. This is not so with a fish deprived of all his otoliths or with all his macular nerves severed. Such fish seems to have little idea of the extent of a forward swim. He is often restless and frequently alters the direction of his progression" (Lee,[3]). In his experiments Lee did not include the positive side of the rôle of vision in the orientation and equilibrium of the fish. Lee says, "When left to himself, the blinded fish swims normally in all respects, moving gracefully, easily, and without timidity, and shooting and diving like an uninjured fish."

The first question which confronts us is, of course, the cause and mechanism of the tilting following the eye dislocation. There can be little doubt that the tilting is directly due to the dislocation of the organ for light perception. We avoided injury to the brain and otic capsule and the immediate post-operative behavior of the fish proves that there were no lesions of those organs.

Any continuous tilting of the fish is called a "forced position" by Loeb. Loeb discriminates in one of his earliest papers between two different types of forced responses: "forced movements and forced position." "Wir sprechen von Zwangsbewegungen, wenn die Tiere sich kontinuirlich oder sehr häufig in Bahnen bewegen, die von denen eines normalen Thieres unter den gleichen Umständen in einem bestimmten einfachen Sinne abweichen." And on the other hand: "Wir bezeichnen als Zwangslagen die Abweichungen von der normalen Orientierung gegen den Schwerpunkt der Erde."

According to Loeb the forced *positions* are due to geotropism. Moreover, he refers to the fishes as one of the clearest instances of such phenomena. The normal *position* of fishes in swimming or at rest is also, according to Loeb, a geotropic phenomenon. "Versuchen wir es einen solchen Fisch gewaltsam auf den Rücken zu legen, so widerstrebt er und bringt, so bald wir ihn wieder frei lassen, sich wieder in seine gewohnte Orientierung zurück."

Even the position of the eyes are influenced by the gravitation in the fish. "Bringen wir den Kopf eines Fisches gewaltsam in eine andere als die ihm zukommende Orientierung gegen die Schwerkraft der Erde, so gehen die Augen völlig oder theilweise in die alte Orientierung zurück. . . . Das Licht hat mit diesen Erscheinungen nichts zu schaffen, sie treten auch, wie bekannt, im Dunkeln und bei völlig Erblindeten ein." (Loeb [5]). As Loeb points out the orientation of the fish in relation to gravity takes place by means of the otolith apparatus, as first demonstrated by Mach and Breuer. Loeb cut (in *Scyllium canicula*) the right VIIIth nerve. He saw forced circus movements to the side of the lesion, forced tilting toward the side of the lesion, pleurothotonus, and compensatory positions of the eyeball and fins.

Bethe does not deny the static function of the labyrinth, but he states that the geotropism of the Elasmobranches is not essentially changed by the removal of *one* labyrinth. He failed to observe in many animals the forced positions as after-effects of unilateral labyrinth-extirpation, but admits that forced movements and forced positions of the eyeballs and fins can often be seen in such animals. He says that the unbalanced tonus and innervation of the trunk, eye and fin muscles (Ewald) can explain satisfactorily these forced motions. But he admits that this tonus factor is also influenced by the geotropic function of the labyrinth.

Although different authors describe somewhat differently the after-effects of labyrinth extirpation (this may be due to difference in technique, injury of other cranial organs, etc.), all seem to agree on the point that the *geotropic* function of the labyrinths is responsible for normal orientation and equilibrium.

No other factor has been considered to play an important part in this behavior. Dogfish in which both labyrinths were removed as observed by Maxwell (8), could hardly be seen to differ from that of the normal fish except when greatly excited. They swim quietly around or settle on the bottom in normal position. Maxwell raised the question whether the orientation of labyrinthless fish is due to retinal stimuli, but his experimental results showed to him that visual impulses did not play a rôle in the orientation of his operated fish. When he covered each eye with a large patch of heavy, black, rubber cloth, the animal swam about with good orientation and never came to rest on the bottom in an abnormal position. This goes to show that in nature chronic or permanent tilting of the body of the fish occurs only from unilateral injury of the labyrinths and never from eye injuries. When both labyrinths are removed there remains no mechanism on which gravity can act in the way to induce orientation, according to Loeb.

In our fish the labyrinths were normal and the otoliths should have taken care of the normal position in swimming or at rest. Nevertheless the tilting of the body took place, as described. Evidently the influence of the labyrinth was modified by a new factor of vision. This factor is probably the influence *of the visual field* on the orientation mechanism.

As long as the vision was impaired by the trauma of the operation, no tilting of the body was noticed. The question is, whether this tilting is a phototropic reaction, a "forced" reaction or due to attempts of the animal to keep its usual visual field. Maxwell admits the possibility of such a mechanism, of course in a different type of experiment: "When the dogfish is rotated around any one of these axes the eyes move as if to retain their original position in space, or to preserve the original visual field."

We think that the tilting is due to so-called "voluntary" motor attempts of the fish to regain or retain the *usual* visual field.

Heliotropic reactions involve different mechanism in fish than in the invertebrates, for Parker has shown that the dogfish, when only one optic nerve is cut, never moves in circles.

Since in the tank, where the fish lived, there were diffused light conditions, there was no single source of light that could act as a phototropic stimulus. Interpretation of the tilting as a *simple* phototropic response seems therefore excluded.

The tilting at 45° was the optimal condition for the animals to *see* the walls of the aquarium and most of the environment with which he can come in contact and our results indicate that the tilting is a quick reaction of the organism as a whole.[1]

SUMMARY.

1. The dislocation of one eye into the top of the head and the removal of the other eye produce a tilting of the whole body with its dorsoventral axis 45° to the side of the vertical. The reposition of the dislocated eye into its original orbit changes the orientation and equilibrium of the fish immediately, it regains its normal position in swimming and at rest.

2. The tilting is probably due to the attempts of the animal to keep its usual visual field.

The writers wish to express their thanks to Professor A. J. Carlson for his constant help and encouragement.

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[1] If so, then the fish placed in the dark or by cutting its only optic nerve should regain its normal position. We contemplate to investigate this and related problems in further experiments.

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BIOLOGICAL BULLETIN

MORPHOLOGY AND MITOSIS IN *TRICHOMONAS TERMOPSIDIS*, AN INTESTINAL FLAGELLATE OF THE TERMITE, *TERMOPSIS*.[1]

JUSTIN M. ANDREWS,

Introduction.

In recently published papers by Cleveland ('24 and '25) establishing the symbiotic relationship of termites and their intestinal protozoa, and the effects of starvation and oxygenation upon this association, he mentions that he has observed in the intestine of termites of the genus *Termopsis*, a flagellate possessing "four anterior flagella, axostyle, and undulating membrane" which he calls "*Trichomonas termopsidis*." The object of this paper is to describe the morphology and mitosis of this species as it occurs in the large Pacific Coast termites *Termopsis nevadensis* Hagen and *T. angusticollis* Hagen.

Material.

Sixteen colonies of *Termopsis* furnished the material for this study. Six of the colonies came from Oregon, and ten were from California. Winged forms appeared in two of the Oregon colonies, and were identified as *T. nevadensis* Hagen. Winged forms appeared in five of the California colonies which were identified as *T. angusticollis* Hagen. The insect material was identified by Dr. T. E. Snyder, Specialist in Forest Insects, Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C.

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[1] From the department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University. A portion of this work was done at the Biological Laboratory, Cold Spring Harbor, Long Island, New York.

ACKNOWLEDGMENTS.

Many thanks are due Dr. R. W. Hegner for his valuable suggestions and criticisms. To Dr. L. R. Cleveland I am especially indebted for furnishing the material used, for suggestions as to methods, and for the oversight which he so generously gave.

METHODS.

In order to obtain preparations which contained more than the usual number of the flagellates per unit area, the termites were starved for eight days, at which time the two larger protozoa, *Trichonympha campanula* and *Leidyopsis sphaerica*, which had been previously present in large numbers, had entirely disappeared (Cleveland '25). Then the termites were fed a pure cellulose diet (Whatman Filter Paper No. 43) which resulted in a much greater clarity of the intestinal fluid surrounding the flagellates and relieved the animals of many food inclusions (wood particles) that had previously obscured their internal morphology.

Smears were made from the intestinal contents of termites treated as above. They were fixed in various fluids,—Schaudinn's strong Flemming's, Gilson's, Bouin's, Zenker's, Carnoy's, osmic acid vapor, and chromic acid. These were stained, for the most part, with Heidenhain's iron alum hæmotoxylin; a few were stained with Mallory's Tri-Stain. Others were dry fixed and stained with Wright's stain. Sections of the intestines of termites—both of individuals that had been partially defaunated as above, and of untreated hosts, were fixed in Schaudinn's fluid, Flemming's fluid, chromic acid, and osmic acid vapor. They were stained with iron hæmotoxylin, and with Mallory's Tristain. The live animals were also studied under vaselined coverslips.

MORPHOLOGY.

Shape and Size of Body.

Body shape in *Trichomonas termopsidis* is exceedingly variable, due to the absence of a restraining pellicle, and to the extreme lability of the cytoplasm. From a pyriform shape, it varies through a regular and elongated oval, to a spheroidal contour. The greatest width is usually near the middle of the animal, and varies, in proportion to the length, from 1 to 4.5, to 1 to 1.

The size is also very variable, owing perhaps, to division processes, the figures of which are exceedingly numerous. The majority, though by no means all, of the dividing forms are larger than the resting individuals. Of one hundred measured as resting trophozoites, the length averaged 29.17 micra, and the width 14.49 micra. The extremes of length were 55.0 micra, and 11.0 micra, the majority of the individuals falling between 25 and 35 micra. The extremes of width were 28.8 micra, and 6.78 micra,

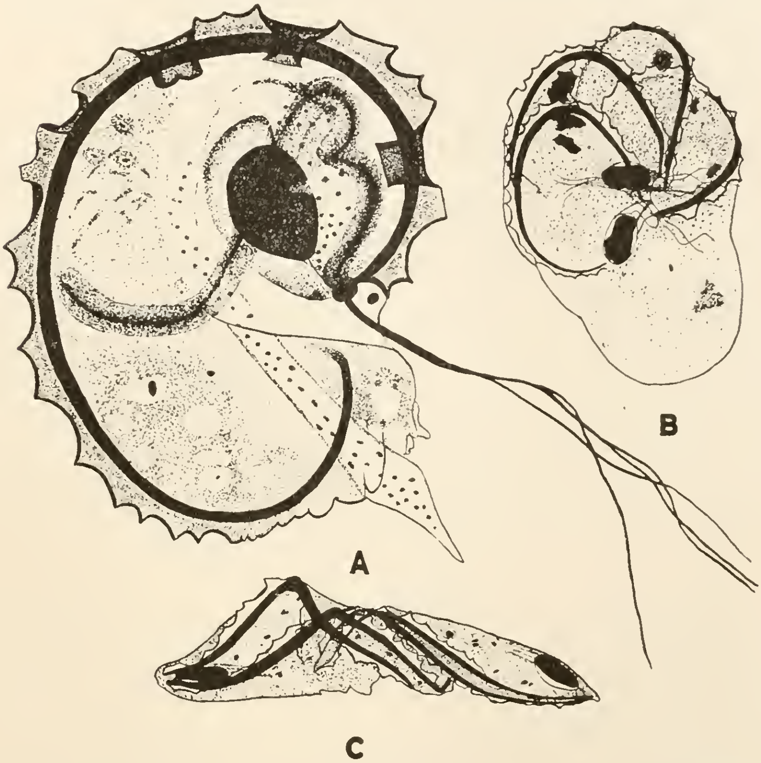


FIG. 1. All figures of *Trichomonas termopsidis* Cleveland, from material stained with iron alum hæmotoxylin, drawn with the aid of camera lucida. Flagella are shown as they appeared in the preparation.

A. Typical trophozoite in resting stage—fixed in 1 per cent. chromic acid. × 2500.

B. Multiple fission, of the type with the nuclei in a central position—fixed in Carnoy's fluid. × 500.

C. Multiple fission, of the type with nuclei at polar extremities—fixed inCarnoy's fluid. × 500.

the majority falling between 12 and 20 micra. As these measurements were made from fixed, stained, individuals from partially defaunated hosts, the length measurement was checked by measuring one hundred live individuals from untreated termites. The average length in this series was 30.97 micra, indicating that the treatment has little, if any, effect on size.

The above measurements, and those mentioned hereafter in the description of organelles, were all made from termites of three colonies which furnished most of the material used in the cytological study. When other colonies (thirteen more) were investigated in order to observe the distribution of *Trichomonas termopsidis*, forms were encountered in twelve of the colonies that differed from those previously studied in size, which varied from 50 micra to 150 micra, most of the giant individuals lying between 70 micra and 90 micra in length. In all other morphological details, the two sizes of this *Trichomonas* are identical as far as we are able to observe. So, for the present, we are calling this a size race of *Trichomonas termopsidis*. It may be that further study will reveal specific differences rather than racial differences.

Cytoplasm.

The cytoplasm, as stated above, is extremely labile; indeed it probably approaches fluidity. The shape is constantly changing, and the body assuming new proportions. Occasionly, in live specimens, one can see the animal slide some of the cytoplasm off the end of the axostyle in drops. The cytoplasm is kept in a state of agitation by the activity of the axostyle, which appears to function as an internal paddle, continually churning up the contents of the body.

Cytostome.

The cytostome (*Cytos.*, Fig. 2.) does not appear to be the comma-shaped vent usually pictured for the trichomonads, but extends as a spiral groove starting above the middle on the right-hand side of the animal, becoming more sharply indented as it proceeds ventrally, ending under the nucleus. Although an actual opening cannot be seen, the food particles, and fluid filled vacuoles are more numerous dorsal to the nucleus indicating the position of the opening.

Nucleus.

The nucleus is an ellipsoidal mass enveloped by a membrane which is sometimes closely applied to the darkly staining nucleoplasm, but is more frequently found surrounding it a short distance away, leaving a clear, unstaining zone between the membrane and the nucleus (Fig. 4, *B* and *C*). Sometimes this zone is not regularly parallel to the contour of the nucleus, but in all such cases observed, the irregularity seems to be due to depressions or elevations in the outline of the nuclear mass, rather than to a collapse of the membrane (Fig. 4, *A* and *D*). The length of the resting nucleus is ordinarily about twice its width. It is always situated anterior to the middle of the body, except in dividing forms, where proportions are upset both in the body of

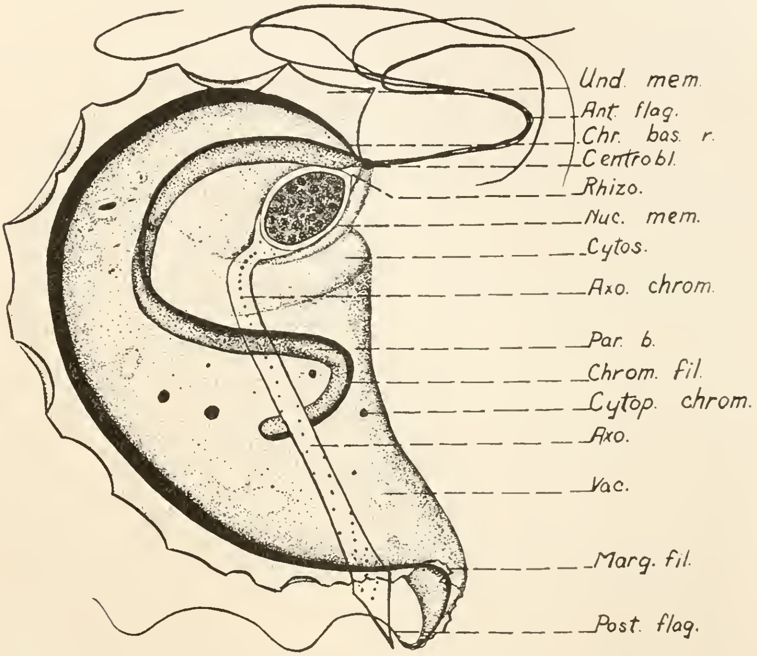


FIG. 2. Diagrammatic representation of *Trichomonas termopsidis*.

Abbreviations: *Und. mem.*, undulating membrane; *Ant. flag.*, anterior flagella; *Chr. bas. r.*, chromatic basal rod; *Centrob.*, centroblepharoplast; *Rhizo.*, rhizoplast; *Nuc. mem.*, nuclear membrane; *Cytos.*, cytostome; *Axo. chrom.*, axostylar chromidia; *Par. b.*, parabasal body; *Chrom. fil.*, chromatic filament; *Cytop. chrom.*, cytoplasmic chromidia; *Axo.*, axostyle; *Vac.*, vacuole; *Marg. fil.*, marginal filament; *Post. flag.*, posterior flagellum. × 3000.

the individual and in the nucleus. Its length varies from 5 to 10 micra. It stains a flat black unless the preparation is strongly decolorized, in which case it shows a granular composition, within which appears a karyosome-like unit contained in a clear unstaining vesicle (Fig. 4, *A*). In the resting stage, the nuclear membrane is connected to the centroblepharoplast by a single rhizoplast.

Neuromotor Apparatus.

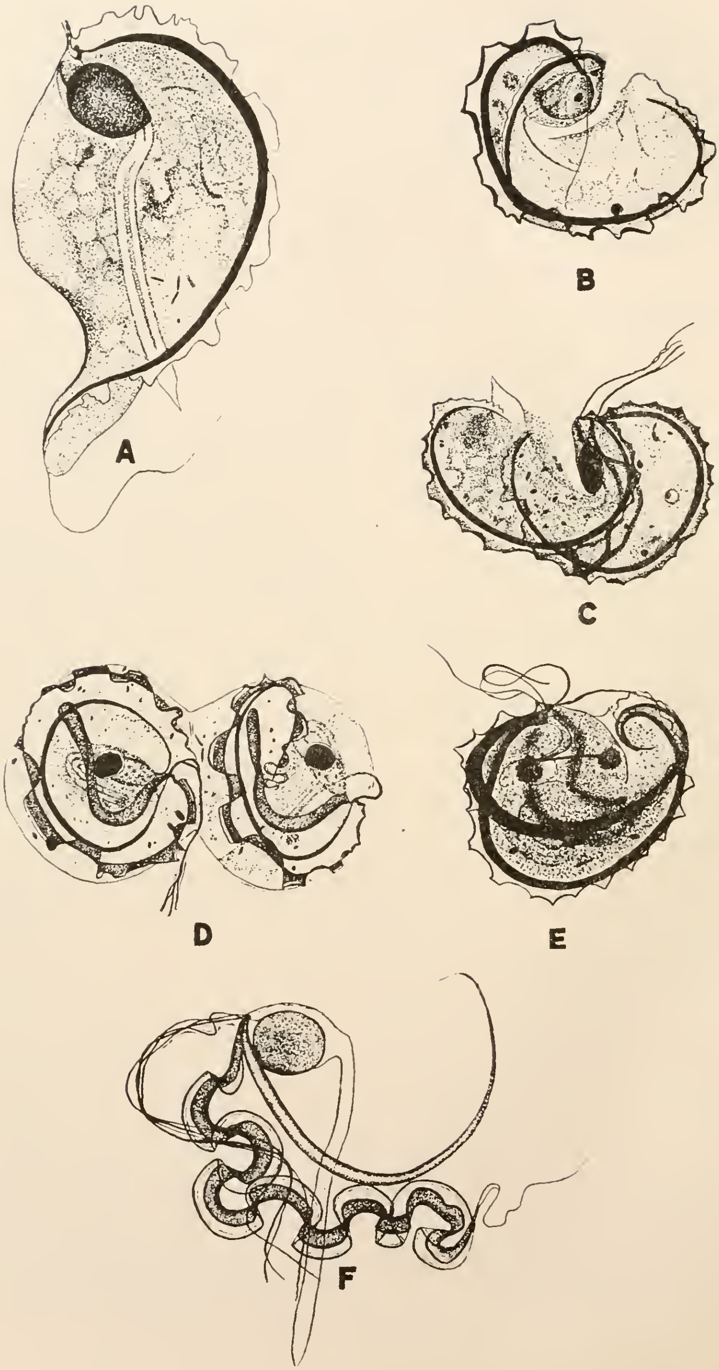
In their paper dealing with *Trichomitus termitidis*, Kofoid and Swezy ('19) list the organelles composing this "complex, structurally integrated apparatus which links together the nucleus and motor organs" as: a centroblepharoplast, the anterior flagella, the undulating membrane, a posterior flagellum, a "parabasal body," and a nuclear rhizoplast. To that category, we would add, in describing *Trichomonas termopsidis*, an axostyle, and would call the organelle referred to as the parabasal body by Kofoid and Swezy, the chromatic basal rod, in view of the fact that a body first called the parabasal body by Janicki ('11), and later corroborated by Alexieff ('13), Kuczynski ('14), Janicki ('15), Cutler ('19), and Wenrich ('21), is plainly demonstrable in our preparations fixed in strong Flemming's fluid, osmic acid vapor, or chromic acid.

The *centroblepharoplast* (*Centro.*, Fig. 2.), apparently a single morphological unit while the trophozoite is in its resting stage, appears as a small, round, blackly-staining dot about a micron in diameter. It is closely applied to or embedded within the anterior end of the chromatic basal rod, so that except during division processes, its position is marked only by a bulbous enlargement of the extremity of the chromatic basal rod. The centroblepharoplast is always connected to the nucleus by one or more nuclear rhizoplasts. From the centroblepharoplast spring the four anterior flagella, the undulating membrane, the chromatic basal rod, the axostyle, and the parabasal body.

The four *anterior flagella* (*Ant. flag.*, Fig. 2) are about equal in length, arise from the centroblepharoplast, and pass discretely out of the cytoplasm. At the point of emergence, the cytoplasm is usually raised to a small hillock. As the flagella leave the body, they are frequently so intertwisted and woven together, that it looks as if a single large flagellum proceeded from the body, and, at a short distance, became divided into four. This illusion seems particularly real as one observes the live specimens, but upon careful examination of the stained preparation, it is obvious that the flagella are not only separate outside the cytoplasm, but within it also. The anterior flagella are about three-quarters the length of the body. Specimens stained by Wright's method show the flagella very clearly, and it was not until this technique was employed that the number of flagella was unquestionably established.

The *undulating membrane* (*Und. mem.*, Fig. 2) is a thin, rippling film, apparently composed of a fold of the pellicular covering, attached, at its outer edge, to a posteriorly directed flagellum, which, like the anterior flagella, originates in the centroblepharoplast. The flagellum separates from the membrane at the posterior end of the body, as an inconspicuous trailing flagellum, which is apparently not as well developed as the anterior flagella. The membrane is hyaline, and persists after treatment which dissolves away the cytoplasm of the animal. It is best developed at the anterior end, and becomes less developed and effective towards its posterior end. In preparations stained by Wright's method, the undulating membrane is differentiated into three distinct zones—a distal, narrow, pink-staining line, which is probably the flagellum, then a pellucid, unstained zone approximately half the remaining width of the membrane, and lastly a heavily staining (pink) zone which extends to the attachment (Fig. 3, Fig. 6). A similar differentiation was observed in several specimens stained with iron alum hæmotoxylin that were more critically decolorized (Fig. 3, *D*).

The *chromatic basal rod* (*Chr. bas. r.*, Fig. 2) extends the length of the body, directly beneath the undulating membrane, in a curve that resembles either a capital "C" or "S." It takes its origin from the centroblepharoplast, and like that organelle, stains intensely black. It retains its stain, ordinarily, after the nucleus has become quite decolorized. Whether it functions as a source of energy for the motor activities of the undulating membrane (Kofoid and Swezy, '15) or serves as a skeletal support for that organelle, the fact is that the membrane seems to be


A
B
C
D
E
F

attached directly to the chromatic basal rod. This observation is strikingly demonstrated in those cases where the cytoplasm of the animal has been destroyed, and the entire neuromotor system with, or without the nucleus, remains intact. In the majority of such cases, though not in every one, the undulating membrane remains adjacent to the chromatic basal rod—suggesting that there may be some connection between them which is strong enough to withstand the treatment that destroys the cytoplasm, but which cannot, in a few cases, endure the chance violences of the smearing operation.

The *axostyle* (*Axo.*, Fig. 2) in *Trichomonas termopsidis* is one of its most conspicuous features when the animal is studied in the living state. As the flagellate moves about, turning and twisting, one of the first things to be noted is the projecting trunk of the axostyle. Occasionly it sticks out of the body for a distance as long as the length of the body, but more frequently it barely protrudes through the cytoplasm, the length of the extruded portion being varied both by changing the actual linear expanse of the organelle—which is accomplished by bending it and curving it within the body—but more often by changing the shape of the body, so that more of the axostyle is included at one time than at another.

When the stained preparation is observed, the axostyle is not such a prominent feature. It does not stain well at all. Frequently it does not project beyond the body line, and in such cases it is easily overlooked. Certain fixing fluids show the

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FIG. 3. All figures of *Trichomonas termopsidis* sp. nov., from material stained with iron alum haemotoxylin, drawn with the aid of camera lucida. Flagella are shown as they appeared in the preparation.

A. Trophozoite in early prophase—blepharoplast just divided, and flagella separating into two bundles. Fixed in osmic acid vapor. × 2750.

B. Prophase, showing new chromatic basal rod and undulating membrane growing out from daughter blepharoplast. Fixed in Schaudinn's fluid. × 1200.

C. Later prophase, showing parabasal body split almost entire length. Fixed in 1 per cent. chromic acid. × 500.

D. Late telophase, all structures duplicated, and animals ready to separate. Note flattened, twisted appearance of parabasal bodies—also the pellucid margin of the undulating membrane. Fixed in 1 per cent. chromic acid. × 1000.

E. Slightly earlier stage of telophase—nuclei still connected by paradesmose. Fixed in Flemming's strong solution. × 1300.

F. Isolated neuromotor system, dry fixed, and stained with Wright's stain. × 1150.

axostyle, after the preparation is stained, much better than others. It is much more apparent in individuals fixed in osmic-acid vapor, chromic acid, and strong Flemming's fluid, than in Schaudinn's fluid, Carnoy's fluid, and Gilson's fluid. It shows very well in specimens stained with Wright's stain. At its anterior end, where it passes under the nucleus, it expands into a capitulum at least twice its lower width, and then tapers rapidly to a point where it is connected to the centroblepharoplast. Passing from its enlarged portion, the axostyle almost invariably takes a bend of at least 90 degrees. The particles of cytoplasm surrounding the organelle at this point stain more deeply than the rest of the proximal cytoplasm (Fig. 1, *A*). The axostyle does not seem to be a cylindrical rod. It is flat, and, at its posterior end, it is sharply pointed. It has been observed in some preparations to be folded back on itself after it has pierced the body. Frequently the trunk is not parallel for its entire length but gradually enlarges from its most narrow portion directly posterior to the capitulum to its maximum width near the posterior end (Fig. 1, *A*). Then it tapers at once to an acute point. In some other cases, the width of the trunk is the same throughout its length.

The granules of the axostyle—axostylar chromidia (*Axo. chrom.*, Fig. 2)—stain intensely, and it is by their aid that the axostyle is recognized more frequently than by its own outline. They are sometimes arranged in a straight line (Fig. 3, *A*), occasionly they are arranged in spiral rows, (Fig. 1, *A*), and not infrequently one finds them scattered throughout the organelle in an apparently hit-or-miss fashion.

The literature concerning the *parabasal body* (*Par. b.*, Fig. 2) is so well reviewed in previous papers (Kofoid and Swezy, '15, Cutler, '19) that it is sufficient to state in this paper that Janicki ('11, '15), Alexieff ('13), Kuczynski ('14), Cutler ('19), and Wenrich ('21) have all reported the presence, in various trichomonads, of darkly-staining, club-shaped organelles—*in addition to* the elongated, slender, chromatoidal rod that lies under the undulating membrane. Kofoid and Swezy ('15) are inclined to regard this as the first step in the origin of the new chromatic basal rod in the prophase of fission. The figures of the developing

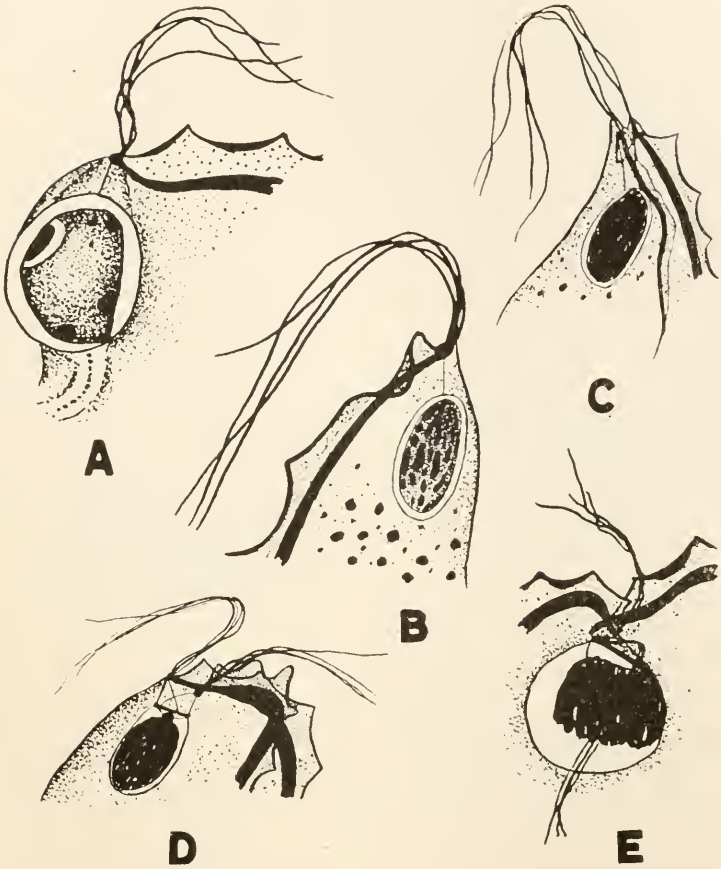

FIG. 4. These figures (Fig. 4 and Fig. 5.) are drawn to show the succession of nuclear changes during mitosis. The chromatic basal rod, undulating membrane, and anterior flagella are also indicated in order to orient the specimen. All material fixed in Schaudinn's fluid, drawn with the aid of camera lucida, and reproduced at a magnification of 2000.

A. Resting nucleus, showing karysome.

B. Chromatin beginning to condense and break up into irregular fragments that tend to arrange themselves linearly. Chromidia appearing in the cytoplasm.

C. Linear arrangement of chromatin more pronounced, centroblepharoplast has released centrosome, which has just divided, as has the blepharoplast and rhizoplast. New chromatic basal rod appearing, accompanied by thin undulating membrane. Cytoplasmic chromidia disappearing.

D. Flagella, chromatic basal rod, and undulating membrane completely duplicated; paradesmose has descended to nuclear membrane.

E. Chromosomes strung over the subparadesmosal fiber—judging from the complex appearance and probable number, they have already divided.

chromatic basal rod published by Kofoid and Swezy ('15), presumably in support of this view, differ from figures published by the other authors purporting to show parabasal bodies, both in size, shape, and spacial relationships.

The parabasal body is an elusive organelle, and is apparently dissolved out by some fixatives, leaving nothing to suggest its shape or position. It is very sensitive to decolorization, and is seldom seen in those preparations which are decolorized sufficiently to permit analysis of mitotic phenomena. But in preparations fixed with chromic acid, osmic acid vapor, or Flemming's fluid—and perhaps others—that are not too strongly decolorized, the parabasal body is a very conspicuous feature of *Trichomonas termopsidis*. If the parabasal is not carefully decolorized, it appears as a stout, club-shaped, serpentine organelle which takes its origin from the centroblepharoplast. After more critical destaining, a darker line appears, that is not always in the center, but which weaves from side to side, suggesting the probability that it does not lie within the parabasal body as is indicated by the previous writers, but is on the outside of it. This line—the chromatic filament (*Chrom. fil.* Fig. 2)—is undoubtedly the "parabasal thread" of Janicki. The body appears to have little rigidity of its own, as it sometimes loops itself back and forth on the axostyle (Fig. 1, *A*), and occasionly surrounds it. The parabasal is about the same length as the axostyle, and about the same breadth, but is otherwise readily distinguishable from it.

MITOSIS.

Mitotic phenomena in *Trichomonas termopsidis* are strikingly different from any of the types described by Kofoid and Swezy ('15) for *Trichomonas*, but are identical with the type described for *Trichomitus termitidis* Kofoid and Swezy ('19). Briefly these are as follows: the nucleus darkens, and shortly thereafter the surrounding cytoplasm becomes charged with chromidia (Fig. 4, *B*) which soon disappear. Then the centroblepharoplast releases the centrosome (Fig. 3, *A*), and the remaining blepharoplast splits into two daughter blepharoplasts. Simultaneously the nuclear rhizoplast and the parabasal body split (Fig. 3, *C*). The centrosome maintains its connection with the two new blepharoplasts by means of rhizoplasts (Fig. 4, *C*). As the blepharoplasts become separated, one remains with the old chromatic basal rod, retaining two of the original flagella, the old undulating membrane, and one of the nuclear rhizoplasts. It completes its locomotor apparatus by growing out two new anterior flagella. The other blepharoplast takes two anterior flagella and the other nuclear rhizoplast, but immediately starts to equip itself with two new anterior flagella, and a new chromatic basal rod, complemented by a new undulating membrane (Fig. 4, *C* and Fig. 3, *B*).

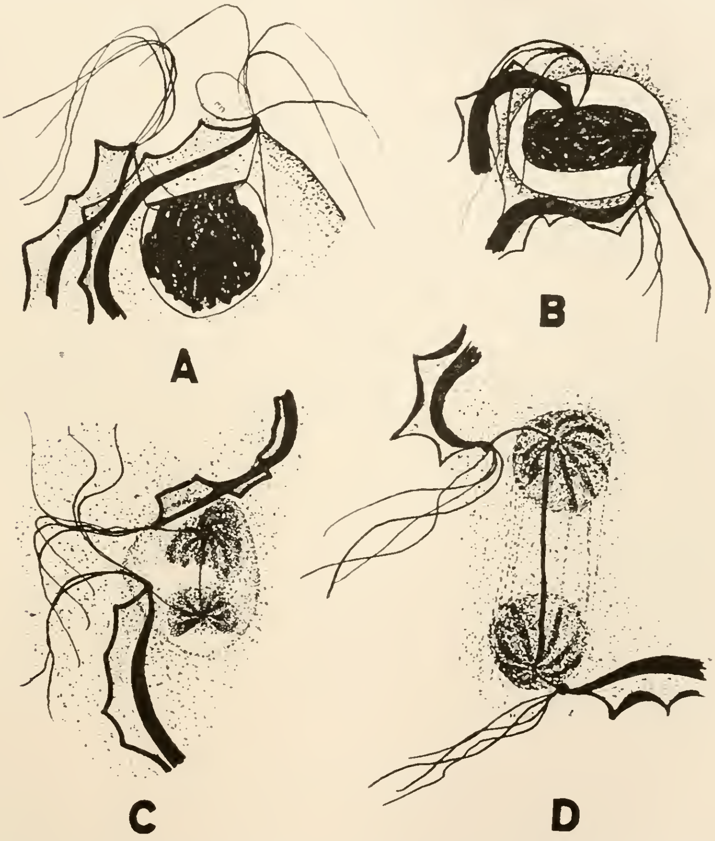

FIG. 5. *A*. Chromosomes have straightened themselves out and are seeking the polar extremities of the paradesmose—they are still on the subparadesmosal fiber.

B. Chromosomes beginning to congregate at the polar ends of the paradesmose.

C. Chromosomes grouped in rosettes at ends of paradesmose, and beginning to pull apart.

D. Chromosomes still in rosette formation, but widely separated, and ready to reorganize.

In the meanwhile the centrosome has split, and the two portions migrated apart, spinning out between them an intensely staining bar, the paradesmose. By the elongation of the rhizoplasts connecting the centrosomes and blepharoplasts, the paradesmose descends to the nuclear membrane. As it approaches the membrane, the chromatin within begins to arrange itself in linear fashion perpendicular to the paradesmose, and shortly after, the paradesmose comes in contact with the membrane, and is greeted by a projection of chromatin that is thrust up from the central mass. At this time, the subparadesmosal fiber appears (Fig. 4, *D*) upon which the chromosomes seem to arrange themselves shortly after (Fig. 4, *E*). Probably the chromosomes divide at this time while they are in this position, for the next figures we are able to find show them being gradually drawn to the ends of the paradesmose, without any indication of spindle formation. The number of chromosomes is difficult to determine, but is somewhere around ten. The chromosomes form irregular clusters or rosettes at the ends of the paradesmose, which always seems to maintain itself as a fairly straight line (Fig. 5, *C*). The two masses of chromosomes continue to migrate apart during anaphase (Fig. 5, *D*) and the paradesmose persists. In telophase, the nuclei assume their characteristic ellipsoidal shape, surrounded by hyaline zone and nuclear membrane, and the paradesmose gradually fades away. The two animals now separate by a plasmotomy, which is materially aided by the activity of the locomotor apparati of the two individuals.

The division of the axostyle we have not been able to observe—but from its capricious staining reactions during prophase, it seems probable that it may be absorbed, and new ones grown out from the blepharoplasts. By telophase, the two axostyles are complete.

Multiple Fission.

Multiple fission seems to occur rarely in *Trichomonas termopsidis*. In the material worked on, fifteen or twenty cases were observed, but they were all on one slide—that is, from the intestinal contents of one termite. There were two types of multiple fission, the one where the nuclei were in a polar position (Fig. 1, *C*), and the other where the nuclei were in a central position (Fig. 1, *B*). These two types occurred with approximately equal frequency. In no case was the somatella composed of more than four prospective individuals, and was always found in the prophase of the second division. These somatellæ were much larger than might be suspected. The greatest and least dimensions of ten of them (nuclei in central position) averaged 80.47 micra and 48.79 micra respectively. The extremes of the greatest dimensions were 111.80 micra and 62.68 micra, and of the least dimensions, 66.91 and 30.49 micra. It is quite possible that these multiple fission figures are related to the giant race of *Trichomonas*—noted above—that was found in some of the termites examined.

Whether multiple fission represents a definite stage in the life cycle of *Trichomonas termopsidis*, or whether it is simply a chance happening stimulated by some particular complex of circumstances, we do not know. It is significant, perhaps in favor of the latter hypothesis, that multiple fission was found only in one termite.

Cysts.

Nothing was found on any preparations which could be identified with certainty as being the cysts of *Trichomonas termopsidis*. The protozoön is probably transferred from host to host in its vegetative phases.

Relationships.

This flagellate has been assigned to the genus *Trichomonas* on purely morphological grounds—that is, because of its possession of a cytostome, four anterior flagella, an undulating membrane, a chromatic basal rod, an axostyle, a parabasal body, and a centroblepharoplast.

Its nearest relatives appear to be *Trichomonas trypanoides* Duboscq and Grasse, and *Trichomitus termitidis* Kofoid and Swezy. It is difficult to state all the points of difference of *T. termopsidis* from *T. trypanoides* because of the scantiness of the description of the latter (Duboscq et Grassi '24.). But it is certain that there is a difference in size—"Les *T. Trypanoides* de courbure normale ont une taille assez constante de 16 micra." It is also found in a host, *Reticulitermes lucifugus*, which belongs to a different family (*Rhinotermitidæ*) from that of *Termopsis* (*Kalo-*

termitidæ). And finally, in *T. trypanoides*, there is a notable lack of constancy in the number of anterior flagella, which vary from one to four.

Trichomonas termopsidis differs from *Trichomitus termitidis* primarily by the possession of an axostyle. *Trichomitus termitidis* is described from *Termopsis angusticollis*? (Kofoid and Swezy, '19). In our material, we are positive of five colonies of the sixteen studied as being *Termopsis angusticollis*, but it is very probable that of the nine remaining unidentified colonies (two were identified as *Termopsis nevadensis*) some are *Termopsis angusticollis*, as the distribution and frequency of occurence of these two species is the same in Oregon and California (Banks and Snyder, '20). In mitosis, *Trichomonas termopsidis* is identical with *Trichomitus termitidis*, which differs, as far as we are aware, from every other form of trichomonad division described. Inasmuch as both forms are found in the same hosts, and as a size race of *Trichomonas termopsidis* agrees in measurements with those given for *Trichomitus termitidis*, and more particularly because the same peculiar type of phenomenon takes place at mitosis in both forms, which has not been described for any other forms, it would appear that *Trichomitus termitidis* should be suitably confirmed before its validity is established.

As *Trichomitus termitidis* differed from its type species, *T. parvus* Swezy ('15) in having the type of division where the centrosome is separated from the blepharoplast during the process, whereas division in the type species was of the kind described in *Trichomonas* and *Eutrichomastix* by Kofoid and Swezy ('15), the species of *Trichomitus* found in the termite was placed in a new subgenus, *Trichomitopsis*. Then, since *Trichomonas termopsidis* differs from the other trichomonads previously described, in the same manner, it is proposed to assign this flagellate to *Trichomonopsis* subgen. nov.

Trichomonas with centrosome separated from blepharoplast at mitosis. **Type Species,** *Trichomonas termopsidis* Cleveland, from *Termopsis nevadensis* Hagen, and *Termopsis angusticollis* Hagen.

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RECENT CONTRIBUTIONS TO THE KNOWLEDGE OF THE CRYSTALLINE STYLE OF LAMELLIBRANCHS.

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The crystalline style of pelecypod and of gastropod molluscs has long been a fascinating subject for investigation by students of these groups. In a paper (Nelson, '18) published six years ago the attempt was made to summarize and to analyse the more important contributions which had appeared up to that time, and by original observation to solve some of the problems regarding the origin, nature, and function of this structure. The conclusions of Coupin, '00, Mitra, '01, and others, that the crystalline style contains strong amylolytic ferments, were confirmed. In addition the style was shown to have at least two other functions. The first and more important of these is the rôle played by the style in the separation of food materials from sand and other waste. The powerful cilia of the style sac spin the style around on its long axis while pushing it anteriorly into the stomach. The head of the rotating style gathers the food strings coming from the œsophagus and as the whole mass is whirled around in the stomach much of the sand and other non-digestible matter is sorted out mechanically by the ciliary tracts of the stomach wall, and passed on into the intestine. At the point where the head of the revolving style comes into contact with the anterior wall of the stomach there is developed a tough resistant covering which I named the "gastric shield" (*flêche tricuspide* of Poli, 1791). The importance of such a sorting and stirring mechanism in animals in which ciliary activity has completely replaced muscular peristalsis, was pointed out.

In forms such as most of our common bivalves, where the style sac is incompletely separated from the intestine by two ridges or typhlosoles, an additional function is served. Food particles escaping from the stomach may be passed across the faces of the typhlosoles from the intestine and then be incorporated into the style. Most of such returned material enters the style sac near its base and is built into the soft core of the style. I have found oysters in which the entire core of the style was almost a solid brown mass of *Glenodinium*. In this position food materials at first rejected from the stomach in the separation of inert materials may be recovered as the style is moved gradually forward into the stomach.

In the interval since this paper, Nelson, *l. c.*, was published there have appeared several noteworthy contributions to the knowledge of the physiology of digestion in bivalve molluscs, and of the function of the crystalline style. It is well therefore to review these at this time and to give such additional information as has been accumulated during the past seven years in order that the present status of the problem of the crystalline style may readily be ascertained.

Edmondson ('20), in a detailed and very well illustrated paper, describes the reformation of the style of *Mya arenaria* following its extraction. Among the species of bivalves which occur on the northwest coast this investigator found in *Cardium corbis*, *Saxidomus giganteus*, *S. nuttallii*, and *Paphya staminea*, that starvation or removal from the water resulted in dissolution of the style "within a few days at most." In other species, notably *Siliqua patula*, *Schizothærus nuttallii*, *Macoma nasuta*, the style was found to be far more resistant to dissolution, being present even at death through starvation. In *Mya* only slight dissolution of the style occurred even after 14 days out of water. (Contrast this with the oyster, in which dissolution of the style occurs while the molluscs are exposed during the low tide; Nelson, '18.) As Edmondson points out, the forms with resistant styles possess a style sac nearly or completely separated from the intestine.

Experiments were performed with *Mya* in which the mantle was cut in the midline along the ventral surface for a distance of 15 to 25 mm. posterior to the pedal opening. A transverse cut was made near the middle of the style sac and the style extracted. The clams were then planted out and the rate of regeneration of the style studied. No food was taken apparently until the style was sufficiently regenerated to project into the stomach, from which Edmondson concludes that ingestion and digestion of food depend upon the degree of development of the crystalline style. A period of approximately 74 days were required for complete reformation of the style following the extraction.

This part of Edmondson's work and the conclusions he draws from it are open to some objection. As the experiments were carried out, there is no way of distinguishing the time actually required for style regeneration from the period of inactivity resulting from the effects of the operation. Out of 147 clams operated upon of which the observation period was in excess of three weeks, only 61, or approximately 42 per cent, survived the operation. Any operative procedure which results in the death of over one half of the animals should be carefully checked before conclusions are drawn from the results, and it seems that such checks were not made. The cut through the mantle alone must seriously have interfered with feeding. Bivalve molluscs are very sensitive to injury and will "sulk" without feeding for long periods after even slight disturbances.

A much better method of determining the period required for regeneration of the crystalline style would seem to be to determine the rate at which this structure is pushed forward into the stomach and dissolved during the normal feeding of the mollusc. This may be determined readily in forms which like the oyster and fresh-water mussels have a style sac incompletely separated from

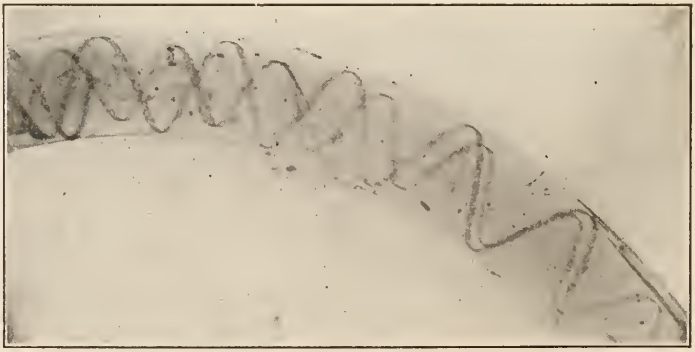

FIG. 1. The crystalline style of *Lampsilis luteola* taken from an individual which had been allowed to siphon in water containing a suspension of fine carmine grains. The spiral bands are composed of carmine which was caught upon the gills, carried into the stomach, thence to the intestine from which it was fed across the typhlosoles at two points near the anterior end of the style sac and incorporated into the style.

the intestine. A weak suspension of finely divided carmine if added to a water rich in food materials will usually be ingested along with the food and may be incorporated into the style. In some instances the carmine is passed across the typhlosoles into the base of the style sac and results in a red style. In others, the carmine may be fed into the style sac at some point between the stomach and the base of the sac, in which case the carmine forms a spiral band about the style as it is rotated and pushed anteriorly, giving the effect of a barber's pole (Fig. 1). Allen ('21) records a similar observation in a fresh-water mussel where minute green organisms took the place of the carmine.[1]

Complete regeneration of the crystalline style in *Ostrea*, *Modiolus*, *Anodonta*, and *Lampsilis* will take place at summer temperatures in from approximately 15 minutes in the oyster to a few hours in the other genera. Allen ('21) finds style regeneration in fresh-water mussels in about 24 hours. It is difficult to believe that a process which occurs in at the most a few hours in many of our common bivalves should require two and a half months in an active rapidly growing mollusc such as *Mya*. Although I have had no experience with this form, it would seem possible to inject by means of a hypodermic syringe a small amount of carmine in sea water near the base of the style sac and to determine the speed at which this pigment, incorporated into the style, is carried toward the stomach. If carefully performed such an operation should be far less drastic in its effects than was the technique employed by Edmondson.

In their admirable work on the natural history and propagation of fresh-water mussels Coker and his associates ('21) accept the conclusions of my 1918 paper, but contribute no original observations regarding the function of the crystalline style. Included in the former paper, pp. 88–91, are the observations of Dr. Franz

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[1] While this paper was in press I received a copy of the report of Dr. J. H. Orton '24 on the causes of unusual mortality among oysters on English oyster beds; Ministry of Agriculture and Fisheries, London. On page 54 of this report is figured the crystalline style of *Ostrea edulis*, bearing a spiral band of food organisms. In a footnote on the same page this author suggests that one function of the style is "the mechanical one of drawing the mucous strings enveloping the food material into the stomach by twisting the strings around the shredded revolving head of the style like a capstan." In my 1918 paper, pg. 101, it is observed that "so strong is the tractive force of the rotating style that strings of mucus from any part of the body if led to the stomach cavity, are at once drawn in and wound up in the food mass."

Schrader on the food of mussels. This investigator found that only about one half of the diatoms and green algæ taken in were digested, and he concluded that these organisms play a comparatively unimportant rôle in the food supply of mussels. He apparently was unaware that in the process of sorting over of the acquired food materials by the ciliary tracts of the stomach, aided by the rotation of the crystalline style, many food organisms are shunted off into the intestine along with the sand and dirt. Especially may this be true of relatively heavy forms such as the diatoms. Some of these escaped food organisms may be passed across the typhlosole into the style sac and thus eventually be returned to the stomach, but a considerable number escape undigested from the anus. Blegvad '15 likewise draws false deductions as to the "insignificant" rôle played by plankton organisms in nutrition, from the finding of living planktonts in the fæces of the European oyster.

While at Madison, Wisconsin, I kept *Anodonta* and *Lampsilis* for months at a time in clear running water in which the chief food supply consisted of desmids, diatoms, and nannoplankton forms most of which were growing upon the sides of the tank and upon the shells of the mussels themselves (Allen, '14). Examination of the intestinal contents revealed many living organisms but also the empty tests of numerous diatoms which had been digested. Imperfect as may be the mode of separation of food from dirt by the ciliary mechanisms within the alimentary canal of bivalves, the wonder is that it functions as efficiently as it does. Such living organisms as are cast out in the fæces are not altogether lost since they accumulate on the shells or upon nearby objects, where they multiply rapidly within the fæcal remains and form a rich growth which is continually contributing its quota of food to the siphons of the mollusc (Allen, '14; Martin, '23).

Allen ('21) gives a detailed account of experiments on the effects of various food organisms in the formation of the crystalline style of fresh-water mussels. The details of this work cannot be discussed here, but in general they confirm and extend the findings of his preliminary paper (Allen, '14) and my own conclusions (Nelson, '18) regarding the rôle of food in style regeneration. He further showed that nannoplankton is relatively more effective than is net plankton in effecting style formation.

The only essential difference between Allen's results and mine is that in fresh-water mussels he found that relatively little escaped food was fed back into the stomach by way of the style, and he concluded that this reclaiming function of the style was of relatively little importance in these animals. The largest amounts of such material were found in the core of the style soon after regeneration of a new style had begun, an observation which I can confirm in *Anodonta grandis*. The relative efficiency of this retrieving mechanism in various bivalves will have to be extensively studied before we shall be in a position to determine its importance to molluscs generally. The fact that *Mya*, *Teredo*, *Martesia* and many other genera with a style sac nearly or quite separated from the intestine are able to exist without such a mechanism indicates that on the whole it plays perhaps a minor rôle in nutrition. Phylogenetically it probably represents the development of a strong recurrent tract of cilia situated in the posterior part of the stomach of the ancestors of present-day types; such a group of cilia as may, for example, be demonstrated in the stomach of the larval oyster.

Allen (*l. c.*) lays still further emphasis upon his conclusion of 1914, voiced likewise in my 1918 paper, that the crystalline style arises in response to the presence of food in the stomach. Evidence that such may not be the case is discussed further on in connection with the findings of Berkeley ('23).

Nogouchi '21 examined the crystalline styles of various marine bivalves and gastropods for *Cristispira*, a large active spirochæte which was first discovered in the style of the oyster. The spirochætes were found most frequently in *Ostrea*, next in *Mya*, then in *Modiolus;* but not at all in *Venus*, *Ensis*, *Mactra*, *Mytilus*, *Pecten*, *Fulgur*, and *Nassa*. Gross, however, has reported *Cristispira pectenis* from the crystalline style of *Pecten*. Nogouchi did not know of my 1918 paper in which is discussed briefly the occurrence of *Cristispira* in the styles of certain bivalves and its absence from others. Nogouchi observed that the style of the oyster quickly liquefied after extraction from the body, and that only oysters freshly removed from natural conditions contained this structure. He believes that the great abundance of *C. balbiani* in the oyster is due to the relatively soft consistency of the style in this mollusc, and that the absence of spirochætes in many molluscs is due to the very firm and resistant styles which they possess.

Although the solidity and resistance to dissolution of the style may effect the distribution of *Cristispira*, they are certainly not the most important factors. As Nogouchi himself admits, and as Edmondson showed, the style of *Mya* is very firm and relatively resistant to dissolution, yet it harbors *Cristispira* in an abundance second only to that of *Ostrea*.

Martin ('23), studying the relative importance of the net plankton and of nannoplankton in the food of the oyster, found that water from which even the smallest nannoplankton organisms including bacteria had been removed would, if well aërated, permit reformation of the style in *Ostrea*. He concluded that although the appearance of the style in this mollusc is usually correlated with the taking in of food, this structure may arise in the complete absence of food, presumably as a response to the act of siphoning.[1]

Three months after the publication of Martin's paper appeared an interesting communication from Berkeley ('23) regarding the function of the crystalline style as a possible factor in the anaërobic respiration of certain marine molluscs. This investigator attempts to account for the continued production of carbon dioxide by marine molluscs kept under anaërobic conditions, as demonstrated by Collip ('21). In seeking a possible explanation of this production of carbon dioxide Berkeley tested the reactions of various tissues and of the style of *Saxidomus* with an alcoholic solution of gum guaiacum. Only in the case of the style did he obtain a deep blue color, which suggested to him that this structure might be associated with anaërobic respiration. Molluscs kept under anaërobic conditions showed in all cases an absence of the style. In an earlier paper (Berkeley, '21) it was shown that a disappearance of glycogen accompanies anaërobiosis in *Saxidomus gigantea*, though not in *Paphia* nor in *Mya*.

It is pointed out in a footnote of Berkeley's ('23) paper that the work was done in ignorance of the publications of Mitra, Allen,

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[1] Although an oyster has no siphons, this term has come into such general use for the process of passing water through the gills of a mollusc that it seems best to employ it here—especially as no good substitute appears to be available.

Nelson, and Edmondson, and that in the light of the findings of these and of other workers a more critical series of experiments is needed to determine the relative importance of food and of oxygen in determining style formation. Berkeley concludes that the presence or absence of the style depends upon the presence or absence of oxygen, and he makes the astonishing assumption that food plays no part in the building up of this structure, on the ground that oatmeal added to the water caused no regeneration of the style. No examinations were made to determine whether the animals were eating the oatmeal, nor were any tests made using the natural plankton food of the molluscs.

Taking the results of Martin and Berkeley together, however, I believe that Allen and I were mistaken in laying undue emphasis upon the rôle of food in stimulating style secretion. Although the presence of food in the stomach may play a part in the mechanism of style formation, Edmondson's finding that no food was taken by *Mya* until after the head of the regenerating style protruded into the stomach; Martin's results in obtaining style regeneration in aërated water devoid of all net and nannoplankton; and Berkeley's conclusion that no style forms under anaërobic conditions, all point to the probability that secretion of the crystalline style may be a direct response to siphoning, regardless of whether the incoming water contains food organisms or not.[1]

The chief criticism centers about the following conclusions of Berkeley; first, his assumption that since the style disappears when the bivalve is kept under anaërobic conditions it therefore represents a reserve of oxygen. As well might one conclude that all secretions contain reserve oxygen since secretion is diminished during decreased activity of the organism. Second, no correlation was shown between the size and persistence of the crystalline style on the one hand and the degree of aëration of the environment on the other. As bearing upon the first of these assumptions Gray, '23, showed that for ciliary movement the degree of mechanical activity exactly parallels the relative amount of oxygen absorbed. Since the formation and movement of the style are so largely dependent upon the activity of the powerful cilia of the style sac, it is to be expected that no style would form in an absence of oxygen. From what we know also regarding the oxygen consumption of the glands of mammals it may be assumed that relatively little secretion of style substance would occur in an absence of oxygen. Berkeley's work is open also to the objection that nowhere does he mention having watched his molluscs to determine whether they opened up and siphoned in the anaërobic water. Such observations are imperative owing to "sulking" on the part of some bivalves after handling, even when the surrounding water is plentifully supplied with oxygen and food. I find that the oyster will not open in oxygen-free water until it becomes too weak to remain closed. Dissolution of the style occurs when many species of bivalves remain tightly closed for a few hours, irrespective of whether the surrounding water is aërated.

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[1] Orton, '24, l.c., pg. 55, observes that sound *O. edulis* will reform a style in the absence of food, but from the text it is not clear that all nannoplankton was removed from the water.

In connection with the relation between the degree of aëration of natural waters and style formation it is of interest to compare the crystalline style of *Pisidium idahoense* with that of *Mactra*. *Pisidium* occurs in abundance in the mud at the bottom of Lake Mendota, Wisconsin, where for two thirds of the year the water may be completely devoid of oxygen (Birge and Juday, '11, Cole, '21). *Mactra* on the other hand lives in or close to the breaker line along sandy coasts, where the water at all seasons of the year is saturated with oxygen. The style of *Pisidium* is no larger than that of other Cyrenidæ which live in relatively well aërated creeks and ponds, whereas the crystalline styles of all *Mactra* which I have examined are relatively large, firm, and among the best developed of the styles I have found in any bivalve.

In *Ostrea* the direct relationship of crystalline style secretion to feeding can readily be demonstrated since this process assumes a somewhat rhythmic character. During the flood tide when the bivalves are feeding actively the style is large and firm, but by the late ebb tide, at which time most of the sand has been sorted out and removed from the stomach and digestion is well under way, the style may be reduced to a soft amorphous mass of jelly. The crystalline style is usually thin or entirely lacking at sunrise before active feeding of the molluscs has commenced. I agree therefore with Berkeley that his conception of the respiratory function of the crystalline style rests upon insecure evidence; and I believe that this theory, interesting though it be, must be added to the long list of the purely suppositious functions of the style which have been proposed during the past two hundred and thirty years.

Bökmann ('23) figures the crystalline style and the style sac of *Mytilus chorus* and his account agrees with the conclusions of List and of Mitra. From a histological study of the ciliated epithelium of the style sac he concluded that this must serve to put the style in rotation, although he did not observe the movement. This author apparently was not familiar with the work of recent American investigators.

A paper of much interest and valuable for its collection of many observations under one head is that of Yonge ('23) in which for the first time is given in one place an adequate account of the mechanisms of feeding and of digestion in a bivalve mollusc. This investigator traces the fate of food particles from the time they enter the incurrent siphon until the waste is expelled from the anus. No new information regarding the function of the style is given in this paper, but the recent literature is well summarized and a clear description is given of the rôle of the crystalline style in digestion in *Mya*. A study is made of the effects of varying concentrations of enzyme and of substrate, using the style and starch solutions. The optimum temperature for the reaction was found to lie at approximately 32° C., with complete destruction of the enzyme at 51° C. From the data thus collected it is concluded that the powerful amylolytic ferment of the style of *Mya* shows all of the characteristic properties of such an enzyme.

It is pointed out that the lamellibranchs may be arranged into taxonomic groups on the basis of the morphology of the style sac and intestine, but that such a grouping does not follow the modern classification based on the gill structure. Yonge considers that either our present system of classification is faulty or that independent evolution has occurred within the digestive system. I believe the latter to be the case since the morphology of the digestive tract is to a high degree correlated with the food habits and the habitat of the molluscs. This aspect of the problem is discussed likewise by Robson ('22) for the style sac and intestine of gastropods as well as of lamellibranchs.

Criticisms of Yonge's work are, first, his failure to consider the important rôle of the leucocytes in digestion, especially of fat;[1] and second, an inadequate appreciation of the part played by the hepatopancreas in the digestion and absorption of food as demonstrated by List ('02). From microscopic examinations of this organ during the later stages of digestion in *Ostrea* and especially in *Modiolus* I am led to believe that in these forms even more food is digested in the hepatopancreas than in the stomach. I believe the functions of the stomach, in some lamellibranchs at least, to be chiefly those of sorting over of food materials and the final separation of dirt, and of mixing food and enzymes, rather than those of an organ where complete digestion is effected.

Lazier ('24) in a valuable paper on the morphology of the digestive tract of *Teredo navalis* confirms for *Teredo* the conclusions of my 1918 paper regarding the origin and function of the crystalline style. His investigation of the morphology of the stomach, style-sac, and intestine shows that these structures are essentially similar to those of other molluscs in which the style-sac is completely separated from the intestine. The style of *Teredo* is believed to be rotated in the manner I described for *Modiolus*, and in *Anodonta*, although the actual rotation was not observed by Lazier.

In some of the marine borers, however, (*Pholas* and *Martesia* for example) the style is of such proportions that if rotation occurs it must be very slow. In my 1918 paper is figured a transverse section of *Martesia* showing a style of a mass approximately equivalent to the entire remainder of the body exclusive of the gills. This aspect of the problem needs further investigation, especially as to the part played by the style in the acquisition and possible sorting over of the borings ingested by *Teredo* and its wood-boring allies.

Churchill and Lewis ('24) make a valuable contribution to our knowledge of the mechanism of feeding in young bivalves. No new data are given regarding the nature of use of the crystalline style, the authors accepting the conclusions of my 1918 paper.

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[1] A recent paper by Vonk '24 gives a good summary of the work in this field and adds some original observations.

In conclusion it is apparent that the main facts regarding the origin, nature, and function of the crystalline style may be considered as quite firmly established and that we are now in a position to attack with the aid of modern methods and the newer knowledge of general physiology some of the problems of nutrition in molluscs.

Summary.

The period of 74 days required for style reformation in *Mya* as determined by Edmondson ('20) represents not only the time during which a new style is regenerating after extraction, but also the period of recovery following the rather drastic operation performed by this investigator. From data procured from other species of lamellibranchs it would appear that the time necessary for actual reformation of the style is much less than the figure given.

It was shown (Nelson, '18) that owing to the imperfect mode of separation of food particles from dirt and sand in the stomach of bivalve molluscs, some undigested food materials may escape down the intestine. In those forms in which the style sac is incompletely separated from the intestine a part of this rejected food material may be incorporated into the crystalline style, thus eventually being returned to the stomach. The remainder pass out undigested in the fæces. Failure to recognize this fact has led at least two recent investigators to conclude that such living organisms as are recovered from the fæces cannot be utilized by the molluscs as food.

The degree of solidity and of resistance to dissolution of the crystalline style are suggested by Nogouchi ('21) as factors controlling the presence or absence of Cristispira. That the solidity and the resistance of the style are not the primary factors involved in the distribution of Cristispira is shown by the presence of these spirochætes in the styles of *Ostrea* and of *Mya* which represent respectively minimum and maximum hardness.

Martin ('23) showed that a style may arise in *Ostrea* as a direct response to siphoning in aërated water even when the incoming water is devoid of all net and nannoplankton or other sources of

food. Berkeley ('23) found no style reformation under anaërobic conditions. His conclusion, however, that the style represents a reserve of oxygen is open to serious objection as herein explained.

The conclusions of Edmondson, '20, Allen, '21, Coker and others, '21, Bökman, '23, Churchill and Lewis, '24, and of Lazier, '24, agree in confirming the work of Coupin, '00, and the conclusions of my 1918 paper. Except in so far as the latter are modified by the findings of Martin and of Berkeley, as set forth in this paper, it is believed that the conclusions of Coupin and of the writer represent the consensus of opinion today regarding the origin, nature, and function of the crystalline style of lamellibranch molluscs.

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THE CONDITIONS OF ACTIVATION OF UNFERTILIZED STARFISH EGGS BY THE ELECTRIC CURRENT.

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While sensitivity to the electric current seems universal in living matter, its degree varies greatly—apparently in correspondence with the wide variation in general irritability. It is most highly developed in rapidly responding tissues such as muscle and nerve; but it can be shown to exist in supposedly insensitive cells like epidermal cells, which respond to electrical stimulation by increase of conductivity.[1] Specialized sensory receptors (retina, auditory or chemical senses) all respond to the electric current as well as to their own appropriate forms of stimulation. Electrical sensitivity thus appears to be the primary form of sensitivity.[2] In general, as the work of Nernst and his successors has shown, it is intimately connected with polarizability, which is dependent on the presence of diffusion-resisting or semipermeable partitions enclosing or pervading the protoplasmic system. Evidently the electric current acts within the living system by influencing the chemical reactions at its polarizable surfaces, as in electrode action in general; in living protoplasm the surfaces concerned are those of the protoplasmic structures, and especially of the films delimiting or separating the protoplasmic phases.

The directive or stimulating action of the electric current on growth has been demonstrated in a number of cases, although much remains to be done in this field. The unfertilized egg-cell, however, seems usually to be relatively insensitive to the current. The earlier evidence of electrical parthenogenesis in the eggs of marine animals is inconclusive. Schücking claims to have activated starfish eggs by passing the current from two chromic acid elements for one to two minutes;[1] but the conditions (nature of electrodes, distance apart, quantity of sea-water, etc.) are not described. He does not consider the possibility that acid or alkali electrolytically produced, or heat, rather than the electric current as such, may have been responsible for the effect; and in the light of our own experiments it seems highly probable that this was the case. Schücking found induction shocks to be ineffective, and we have repeated and confirmed this observation. In the condenser-like arrangement used by Delage,[2] the actual physical conditions were ill-defined. Delage's aim was to affect the eggs electrostatically (by induction); they were placed in a layer of sea-water separated by a thin sheet of mica from a sheet of tinfoil, the sea-water and the metal being connected with the poles of a battery. His results were irregular, and he himself expresses doubt as to the real nature of the conditions. The presence of currents and of products of electrolysis seems not to have been excluded.

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[1] Ebbecke, U., *Arch. ges. Physiol.*, 1922, CXCV., pp. 300, 324.

[2] For a fuller discussion *cf.* the recent volume of R. S. Lillie, "Protoplasmic Acti and Nervous Action," University of Chicago Press, 1923, pp. 273 *et seq.*

In the eggs of amphibia McClendon was able to start cleavage and development by passing the alternating current from the lighting circuit (110 volts, 60 cycles) through the water containing the eggs.[3] In this case a current of considerable intensity acted for a brief period (1 to 2 seconds), and the effect was probably not caused by electrolytic products or heat. Further and more precise investigation of the conditions of electrical parthenogenesis in these eggs seems desirable.

Our aim in the experiments described in this paper has been (1) to ascertain whether in fact activation of starfish eggs by the direct electric current is possible, and to what degree, and (2) to determine more precisely the conditions, more particularly of current intensity, time of exposure and temperature, under which the effect is produced. The experiments were performed on the unfertilized eggs of *Asterias forbesii* at Woods Hole during the summers of 1922 and 1923. The direct current was used in all cases. Usually the eggs were exposed to the current during the interval between the breakdown of the germinal vesicle and the separation of the first polar body (prematuration period);[4] for comparison a number of observations were made with fully mature eggs. The procedure was simple; the current was passed through a shallow layer of sea-water containing the eggs, and at regular intervals portions of eggs were transferred to dishes or watch-glasses containing sea-water. Afterwards they were examined. Fertilized and unfertilized controls were kept in all cases.

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[1] Schücking, A., *Arch. ges. Physiol.*, 1903, XCVII., 86.

[2] Delage, Y., *Arch. zool. expér. et gén.*, Sér. 4, 1908, IX., p. xxx.

[3] McClendon, J. F., *Amer. Jour. Physiol.*, 1912, XXIX., p. 299.

[4] The period most favorable for fertilization and artificial activation.

Our preliminary experiments with battery currents of moderate intensity (one to twelve storage cells) gave uniformly negative results, and in all of our later experiments we used the current from the direct current generator of the laboratory. We were not able at first to find suitable electrodes. The ordinary forms of non-polarizable electrodes ("boot" electrodes) proved unsatisfactory because of high resistance and the diffusion of $ZnSO_4$ into the sea-water. When platinum electrodes were used the eggs showed partial activation (membrane-formation) in some experiments; but it could be shown (by first passing the current through a layer of sea-water and then placing the eggs in the sea-water without the current) that this effect was due to—or at least could be produced by—the products of electrolysis. These results indicated the need for an arrangement by which strong currents could be passed through the layer of sea-water without contaminating the latter by the electrode solution or products of electrolysis; and after a number of preliminary experiments the following method was devised. The wires from the direct current line (110 volts) were connected with two broad zinc plates each immersed in a dish of saturated $ZnSO_4$; these dishes with the plates constituted non-polarizable electrodes of low resistance. A rectangular glass vessel containing the sea-water in which the eggs were to be exposed was placed between the dishes. In the experiments of each summer a single vessel was used throughout the whole series; the dimensions in 1922 were 14 x 5.5 x 3.5 cm., in 1923, 13.1 x 6.6 x 3.5 cm. The depth of sea-water was kept constant as nearly as possible throughout each series, 0.4 cm. in 1922 and 0.5 cm. in 1923; the sectional area (from which the estimates of current density were made) was thus 2.2 sq. cm. in 1922 and 3.3 sq. cm. in 1923. The current was conveyed through the sea-water by massive bridges of agar jelly of the same width as the rectangular vessel and connecting the latter with the electrode dishes. These bridges were made as follows: A concentrated solution of agar-agar in sea-water was allowed to solidify in a large beaker; the mass of jelly was then removed and cut into two blocks of the general shape indicated in the figure; one end of each block stood in the $ZnSO_4$ solution and the other in the sea-water, as shown diagrammatically in the longitudinal section (Fig. 1). By means of this arrangement strong currents (up to 2 or more amperes) could be passed through the sea-water. Experiment showed that the composition of the sea-water was not appreciably affected during the flow of the current for the period of an experiment. The temperature, however, rose rapidly unless controlled. In part of our experiments the control of temperature was effected by setting the whole system in a pan of ice water; this method proved satisfactory with currents of moderate intensity (up to 150 ma./cm.).[1] In a number of experiments with stronger currents another method was used, to be described below. The strength of the current was regulated by two rheostats and measured directly by a Weston voltmeter provided with shunts so as to read as a milliammeter over the several ranges required. Intensities as high as 3 or 4 amperes were used for a brief period in some of the experiments with running sea-water described below (densities up to *ca*. 800 ma./cm.).

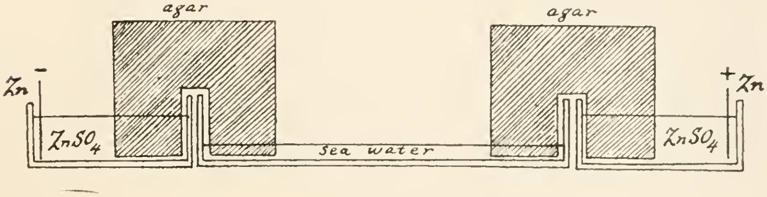

FIG. 1.

The usual procedure was as follows: A somewhat small quantity of eggs was placed with a pipette in the rectangular dish midway between the agar bridges (2 to 4 cm. from each). When the eggs had settled the circuit was closed, and at stated intervals, usually 2, 4, 8 and 12 minutes, successive portions of eggs were transferred to small dishes (usually Syracuse watch glasses or stender dishes) containing sea-water; these were kept covered except at times of examination, and the sea-water was changed several times. With small numbers of eggs this method is satisfactory and convenient. In each experiment the temperature of the sea-water in the rectangular dish was recorded at the end of the longest exposure. The temperature was measured by a thermometer with the bulb placed near the eggs. A difficulty with this method is that the bulb was incompletely immersed when the layer of sea-water was shallow, as in most of our experiments; this was especially true of the earlier experiments, where the readings were too low and a correction of 1 to 3 degrees was found necessary; with later experiments a small thermometer with a short bulb was used which gave reliable readings.

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[1] Milliamperes per square centimeter of sectional area. The commonly used unit of current-density, δ (microampere, *i.e.*, .001 milliampere, per square millimeter), is one tenth of this unit.

A large number of experiments (more than 50) were performed in which the vessel containing the eggs was immersed in ice water as described above; the current densities used ranged from less than 100 to 318 ma./cm. The general results of these experiments may be summarized as follows.

With currents of densities ranging from 136 to 242 ma./cm., flowing from 2 to 12 minutes, activation was either absent or negligible, provided the temperature remained below 29°. In all cases where the temperature rose to 30° or higher a variable degree of activation, usually incomplete, was obtained; and in some cases a considerable proportion of eggs developed to the swimming blastula stage. In the experiment showing the most striking effect of this kind (Aug. 22, 1922) a very typical picture of heat activation was presented; after 4 minutes exposure to a current of 227 ma./cm., only a few eggs (*ca.* 3 per cent.) formed membranes; with exposures of 8 and 12 minutes almost all formed membranes and a large proportion formed blastulæ (*ca.* 75 per cent. with 8 minutes and 25 per cent. with 12 minutes). The temperature at the end of the maximum period of exposure (12 m.), allowing for the error of measurement, was 30° or over. In such a case the activation caused by the current is mainly if not entirely an effect of the high temperature, and not of the current as such. This is shown by the fact that activation was never produced by the same current at lower temperatures (28° or ower); also by control experiments in which eggs were activated by sea-water which had been warmed to 30° or higher by the current, the latter being shut off before the eggs were introduced.

It seems probable, nevertheless, that a part of the activating effect observed in this and similar experiments is to be attributed to the current; *i.e.*, that there is a summation of the effects of heat and current, since the degree of activation was greater than would usually be produced by exposure to a temperature of 30° for the periods used. According to earlier observations, activation by warm sea-water (acting alone) requires a temperature of at least 29°, and at 30° few eggs develop to a blastula stage after less than 15 minutes' exposure.[1] In other words, the effect of high temperature appears to be greater when a current is flowing through the sea-water containing the eggs than when no current is flowing. We have not, however, performed definite controlled experiments to determine with exactitude the degree of this additive effect. An analogous phenomenon is seen in the activation of starfish eggs by fatty acid; at temperatures of 26° and higher the effective times of exposure to the acid are much shorter than can be accounted for by the temperature cœfficient of acid activation (Q_{10} = *ca.* 3.0) shown at lower temperatures.[2] Apparently in warm sea-water the action of the fatty acid is accelerated by some condition dependent on temperature; *i.e.*, there is a superposition of acid activation upon an incipient heat activation. Similarly, in the experiments with strong currents an effect resulting from the action of the current as such appears to be superposed upon that of the high temperature.

With current-densities higher than 240 ma./cm. the difficulty of evading the temperature effect was such that it was necessary to devise another means of compensating the heating action of the current. We therefore tried exposing the eggs to strong currents in *running* instead of stationary sea-water, and after some preliminary experimentation adopted the following procedure: In place of the rectangular glass dish a paper box of the same dimensions was used. This was reinforced and made an electric non-conductor by several coatings of paraffin. A rectangular slit (*ca.* 6 x 1.5 cm.) was cut at the base of this box along one of the longer sides. A small rectangular cloth basket (*ca.* 3 x 3 x 4 cm.), containing the eggs, was inserted between the two agar bridges at the center of the paper-paraffin dish. This basket consisted of a frame of slender wooden sticks reinforced with thread and paraffin, to which silk bolting cloth was sewn. The bolting cloth had 200 threads to the inch—thus confining the eggs, yet allowing a free circulation of water. The apparatus was set on a wooden block in a large pan. A stream of sea water of considerable force was directed through a glass nozzle against the bottom of the paper-paraffin vessel on the opposite side of the basket from the slit. In this way a swift stream of water through the basket was obtained. After passing through the basket the water ran out of the slit down the side of the block into the pan where it was removed with a siphon. A small thermometer was p'aced with its bulb resting on the bottom of the basket.

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[1] Lillie, R. S., Biol. Bull., 1915, XXVIII., p. 260; *cf.* Table II., p. 269.

[2] Lillie, R. S., Biol. Bull., 1917, XXXII., p. 131; *cf.* Table II., p. 142.

In carrying out these experiments the usual procedure was as follows. The sea water and the current were started and the temperature was allowed to reach its position of equilibrium. Then with a pipette a quantity of eggs was placed in the cloth basket, and at the stated intervals the portions were removed to stender dishes of sea water for observation.

The density of current was estimated from the ammeter reading and the average depth of the layer of running water in the paraffined vessel. Under these conditions, even with densities so high as 600 to 800 ma./cm., the current could be passed for several minutes without raising the temperature above 29°, and with lower densities the temperature showed little increase over that of the sea water without the current. In one of the experiments with a strong current (ammeter reading 680–790 ma./cm.) passed for four minutes, the temperature reading was 29° for most of the period of flow but reached 30°–31° for a few seconds toward the end. In a second similar experiment, with a range of 648–810 ma./cm., in which eggs were removed to normal sea water at intervals of $\frac{1}{2}$, 1, 1$\frac{1}{2}$ and 2 minutes, the temperature reading was unfortunately lost; probably 30°–31° was reached in this case also.

With such strong currents the eggs showed marked deformation during the period of exposure, adopting shapes of the kind shown in Fig. 2. This effect is temporary; within a few minutes after removal from the current the eggs return to their original shape. Such eggs show a separation of fertilization-membranes in a considerable proportion of cases, but not in all. In the experiment just cited *ca.* 40 per cent. of the eggs exposed to the strong current for 1½ and 2 minutes showed well separated membranes and a small proportion developed to the blastula stage. The majority underwent disintegration inside the membrane without development.

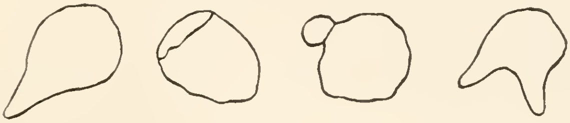

FIG. 2.

Of twelve experiments performed with this method (with good controls), using currents of densities ranging between 130 and 810 ma./cm., seven showed a varying degree of activation. Little or no activation was obtained with currents of less than 300 ma./cm. With higher intensities membrane-formation and activation occurred in a minority of eggs; the strongest currents in addition to deforming the eggs temporarily in the manner just described had a marked destructive effect.[1]

In general the results of the foregoing experiments indicate that the electric current has little activating effect upon the starfish egg unless the intensities employed are sufficient to produce well marked structural changes in the egg-system. The evidence of these changes is deformation and subsequent breakdown of most eggs. A certain proportion of eggs, however, recover and show the usual phenomena of partial activation. These effects cannot be referred to the observed rise of temperature which produces no such definite deformation. Moreover, the highest temperatures reached (30°–31°) require a much longer period of exposure for activation of the degree observed.

It should be remembered in considering the results of such experiments that the physical conditions are far from constant, and that the records of both temperature and current are subject to an error which may be considerable. In general the recorded current density is greater than that to which the eggs were actually exposed, since the current lines extend outside of the rectangular vessel into the overflow. There is also an error of measurement resulting from variability in the depth and contour of the layer of water between the agar bridges; this condition makes possible only an approximate estimate of the sectional areas. The usual procedure was to measure the depth of the water in the basket and on both sides of it and to calculate the cross section from these measurements. But even during a single 5-minute experiment the water level often varied considerably, sometimes because the eggs themselves clogged the silk bolting cloth thus raising the level of water inside the basket. Again, with regard to temperature, although the bulb of the thermometer was completely immersed the irregularity of the water stream is a source of uncertainty. Local temperatures may rise higher than the average temperature recorded by the thermometer; or the instrument may be actually registering the temperature of a stream of water of greater or less velocity than that to which the eggs are actually exposed. It may reasonably be assumed, however, that on the whole the errors in opposite directions compensate each other.

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[1] This destructive action of strong currents on egg cells has been noted by other observers; *cf.* the case of *Crepidula* as described by Conklin, *Jour. Acad. Nat. Sciences*, Philadelphia, 1912, XV., p. 521.

It was thought possible that the physiological effect of the current might be changed (increased or decreased) by changing the balance of salts in the medium. This occurs, for example, in the electrical stimulation of muscle.[1] Eggs were suspended in pure isotonic NaCl solution (0.54 *m*), washed in this solution by gentle centrifuging and decantation, and exposed to the current (densities from 139 to 262 ma./cm.) in the rectangular glass dishes under the conditions already described. A certain degree of activation results from the action of the pure NaCl solution in the absence of the current.[2] The result of passing the current through the NaCl solution containing the eggs was, however, essentially negative; four out of eleven experiments showed a slight increase in activation over that produced by the solution alone, five showed no difference, while two showed a decrease.

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[1] *Cf.* the observations of K. Lucas and G. W. Mines on the electrical stimulation of muscle in *Journ. Physiol.*, 1908, XXXVII., p. 459.

[2] Lillie, R. S., *Amer. Journ. Physiol.*, 1911, XXVII., p. 289.

The combined action of the NaCl solution and the current thus shows no significant difference from that of the pure solution alone. The increase noted in the first four experiments was probably the result of a slight rise of temperature; this increases the activating effect of the NaCl solution, as control experiments showed.

Decreasing the conductivity of the medium by adding isotonic sugar solution to the sea water was also found not to alter the effect of the current on the eggs.

In conclusion brief mention should be made of similar experiments with *Arbacia* eggs. The results of these experiments were mainly negative. Little or no effect was produced by exposing the eggs in standing sea water to current-densities varying from 7.5 to 210 ma./cm. No membranes were formed and no cleavage resulted. Eggs exposed to the current and immediately afterwards treated with hypertonic sea water showed no constant increase in the percentage of activation, above eggs treated with hypertonic sea water alone. The effect of exposing to currents of high density in running sea water was also essentially negative, although some cytolysis was caused by the longer exposures. In general the *Arbacia* egg is more resistant to the current than the *Asterias* egg; this difference is probably to be correlated with the greater impermeability of the surface layer to water[1] (and presumably to water soluble substances) and its greater resistance to alteration and the action of parthenogenetic agents in general.

SUMMARY.

1. A new type of non-polarizable ($Zn–ZnSO_4$) electrode of low resistance is described by which strong electric currents (up to 2 amperes or more) can be passed for prolonged periods through a small quantity of sea-water without appreciably affecting its composition.

2. It was found that unfertilized starfish eggs can be readily and completely activated by moderate currents, of the density 200–300 milliamperes per square centimeter; but that the effect in such cases is due almost entirely to the heating action of the current on the sea-water. When the temperature is kept below 29° such currents produce little or no effect upon the eggs.

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[1] Lillie, R. S., *Amer. Journ. Physiol.*, 1918, XLV., p. 406; *cf.* p. 420.

Unfertilized eggs are thus insensitive to the current as compared with most irritable or active cells of other kinds.

3. When the eggs are exposed in a stream of running sea-water to stronger currents (600–800 ma./cm.) for brief periods ($\frac{1}{2}$ to 2 minutes), definite effects are produced. The eggs undergo marked deformation during the passage of the current, and a variable proportion afterwards show fertilization-membranes and partial activation. Complete activation of a large proportion of eggs was not possible in our experiments, although a few developed to the blastula stage.

4. We conclude that the unfertilized starfish egg is insensitive to currents of moderate intensity, and exhibits activation only when currents are used of such intensity as to produce definite and well marked structural changes in the egg-system.

5. Similar exposure of unfertilized sea-urchin eggs (*Arbacia*) to strong currents, with and without after-treatment with hypertonic sea-water, gave inconstant or negative results.

AN EXPERIMENTAL ANALYSIS OF ASYMMETRY IN THE STARFISH, *PATIRIA MINIATA*

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I. Introduction.

The present paper gives the results of a portion of the experimental work carried on at the Hopkins Marine Station of Leland Stanford University in the spring of 1923. The program of work was a continuation and an extension of the experiments begun in the same laboratory in 1920 and published during the next two years (Newman, '21,*a*; '21,*b*; '21,*c*; '22).

It became apparent in 1920 that not only twinning but other developmental aberrations could be induced by retarding the rate of development of eggs and embryos in a considerable variety of ways. Of all the agents used by various investigators for retarding development, that of low temperatures seemed to be the least open to objection and is perhaps the most readily managed. It was, therefore, with the program in mind, of subjecting *Patiria* and other echinoderms to various degrees of low temperatures at various stages of development and to observe and analyse the resulting developmental aberrations, that work was begun early in April, 1923.

During some of the very first of the preliminary experiments it was found that subjection of *Patiria* blastulæ to temperatures of about 2° C. for two or three hours brought about a marked increase in the percentage of larvæ showing reversed or right-handed asymmetry. For over two months after this discovery it became a major consideration to gather an adequate mass of data on this subject and to work out the implications involved.

Before it was possible to arrive at any justifiable conclusions as to the affects of low temperatures upon asymmetry it was necessary to study in some detail the normal development of *Patiria*. It seems likely that entirely normal development is unattainable under laboratory conditions, yet the culture methods used were experimentally determined to be a vast improvement upon those commonly adopted by experimentors upon like material. As an introduction to the experimental part of this paper we shall ask the reader to make with us a brief survey of the essential features of the normal embryonic and larval periods of Patiria, paying chief attention to the origin and development of asymmetry.

II. The Normal Development of *Patiria*.

Methods of Securing and Handling Eggs and Larvæ.—Patiria miniata is a common Pacific Coast starfish with which I have done considerable work. During the spring of 1920 I had not discovered any better method of securing the eggs than that of shaking the excised ovaries in bowls of sea-water. This method had proven rather unsatisfactory because the great majority of eggs thus obtained were immature and incapable of fertilization even after standing for an hour or so. Furthermore, the presence of so many dying and disintegrating eggs tends to foul the water and to encourage the development of deleterious bacterial and fungoid growths, which are far from favorable for the living and active larvæ. This difficulty was formerly obviated to some extent by taking advantage of the fact that there is a certain period in the early larval life when all healthy larvæ swim to the surface and thus may be decanted off and placed in clean sea water. But many of the slightly subnormal larvæ fail to reach the surface and either have to be picked out individually or left to develop amidst the débris of decaying eggs.

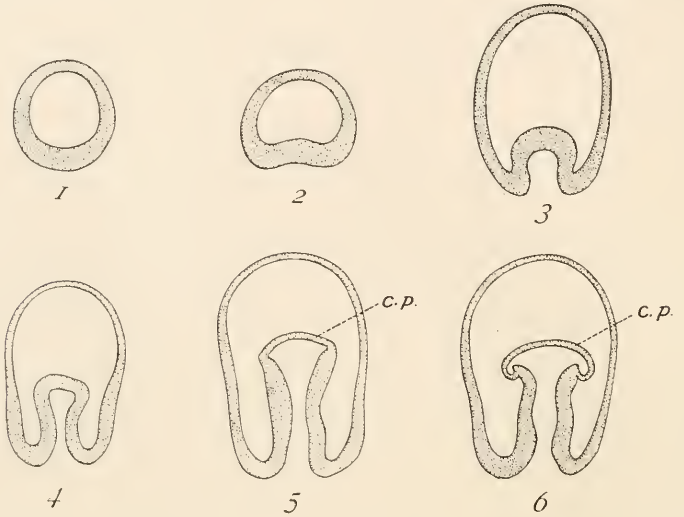
The improved method takes advantage of the chance observation that if ripe starfishes are allowed to stand for a few hours out of water they shed both eggs and milt in great volume. The procedure adopted was to place a considerable number of freshly collected starfishes upon a bed of seaweeds in a moist atmosphere. It is found that about ten per cent. of the individuals shed their genital products within from two to four hours and that both eggs and sperms are uniformly ripe and in prime condition, so that essentially all eggs are fertilized and develop normally. Many thousands of eggs may be shed within a few minutes by a single female, making it possible to conduct several experiments with a single batch of eggs. Variety may be secured by fertilizing one lot of eggs with the sperm of several males. It is necessary to use the minimum sperm concentration in order to avoid polyspermy. The standard procedure adopted and used in all experiments was as follows: Five drops of solid sperm, just as it exudes from the genital pores, was mixed with 100 cc. of sea-water; and then ten drops of this sperm suspension was added to a finger-bowl containing a single layer of eggs and 100 cc. of sea-water. Eggs must stand for about an hour in sea-water before insemination is attempted.

Cleavage, Blastula, and Gastrula.

Cleavage begins after about two hours and is entirely similar to that of other asteroids previously described. To get the best results it is necessary to keep the cultures in an unheated room in which the temperature ranges only a degree or so above or below 15° C. Especially is it important that the embryos should not be warmed; a few degrees of lower temperature may be beneficial. The cultures should also be shaded in such a way that no direct sunlight reaches them. When eggs are reared under these precautions they reach a blastula stage in a little under or over 18 hours, and hatch out as swimmers in about 24 hours. The great majority of the blastulæ are practically spherical in form. Even in the best of cultures there are always a few abnormal larvæ that are wrinkled or nearly solid, but in spite of their deformity apparently more active than normal larvæ. It has not been determined whether these abnormal larvæ are the product of overripe eggs, of polyspermy, or of parthenogenesis. It is a simple matter to rid the cultures of all such abnormal larvæ at an early stage, and thus to make conditions better for the normals.

Gastrulation begins a few hours after the larvæ have hatched, and is in no way different from that described for other asteroids. The various steps in the process are shown in Figures 1–6. It is to be especially noted that gastrulæ reared in this fashion are quite free from any apical thickening such as Heath (1918) described for this species and which he considered homologous with the apical plate of the enteropneustan larva. Only subnormal larvæ show this structure, as was pointed out in a previous paper (Newman, 1922), but this becomes especially obvious when improved methods of obtaining eggs are used.

The completed gastrula is somewhat elongated (Fig. 6) and the anterior end of the archenteron reaches about half way to the apical end. This stage is attained at about thirty hours after fertilization.

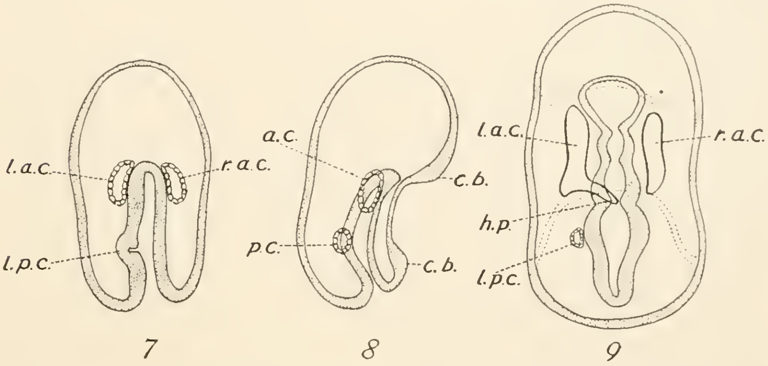


Figs. 1–6. Early stages, blastulæ and gastrulæ, of *Patiria*. *c.p.*, the thinned-out anterior portion of the archenteron in process of forming the cœlomic pouches.

Post-gastrula Stages.—The changes leading up to the typical Bipennaria condition involve several distinct types of differentiation: (*a*) the development of well-defined dorsal and ventral surfaces, together with a relative shortening of the ventral surface, which thus becomes concave, and a relative lengthening of the dorsal surface, which becomes convex; (*b*) the differentiation of the external ciliated bands; and (*c*) the development of the cœloms.

The larva at about fifty hours (Fig. 8) shows the blastopore moving slightly toward the ventral surface, the somewhat convex contour of the latter, the slight thickening and bulging of the region destined to form the preoral ciliated band, and the enlargement and thinning out of the anterior end of the archenteron to form the pharynx and the anterior cœloms. The changes in larval form and the development of the ciliated bands are so nearly identical with those described for other asteroids that they may be passed over, at least until more advanced stages are reached. The events associated with the formation of the system of cœlomic pouches, however, need more detailed treatment for they seem to be unique in certain respects.

Development of the Cœlomic Structures.—As has already been said, the first change leading to the formation of cœlomic structures is the enlargement and thinning out of the free end of the archenteron. This is well shown in Fig. 5. The next stage involves a flattening out mushroom-fashion of this thin-walled vesicle, as shown in Fig. 6. Here the paired anterior cœloms are shown in process of being pinched off. This process completes itself, as shown in Fig. 7, by the end of the third day. I have never been able to assure myself, as other writers have done, that the left anterior cœlom is from the first larger than the right. It appears to me that until the formation of the posterior cœlom the larva is perfectly bilaterally symmetrical.



FIGS. 7–9. Early *Bipennaria* larvæ, showing especially the formation of cœlomic structures. *a.c.*, anterior cœlom; *c.b.*, ciliated band in optical section; *h.p.*, hydropore; *l.a.c.*, left anterior cœlom; *l.p.c.*, left posterior cœlom; *r.a.c.*, right anterior cœlom.

The Posterior Cœlom.—A very significant event, from the standpoint of the development of asymmetry, is the appearance of the posterior cœlomic vesicle. In *Patiria*, this vesicle is a conspicuous object for a considerable period of time and in the end becomes the hypogastric cœlom. This condition is like that described for *Cribrella* and *Solaster* and unlike that in most asteroids thus far described in which the hypogastric cœlom is derived from a posterior outgrowth of the left anterior cœlom. In *Patiria* the posterior cœlom typically arises on the left side of the archenteron at about the level of the middle of the prospective stomach. It is first seen in the form of a thick-walled evagination of the wall of the archenteron (Fig. 7). This pouch does not expand into a thin-walled vesicle, but merely constricts off a small solid ball of cells that later expands and forms a lumen. The vesicle is always a conspicuous object even under low powers of the microscope, and it affords a ready means of determining the asymmetry of the larva before there are any other positive indications of asymmetry.

Variations in the Position of the Posterior Cœlom.—The typical point of origin of this organ is noticeably to the left of the median line of the archenteron. In many cases, however, it occurs exactly in the median line, under which circumstances it is not infrequently completely double or at least more or less constricted down the mid-dorsal line. Sometimes the double vesicle fuses into one and moves sometimes to the right, but usually to the left, and gives rise to right-handed or left-handed asymmetry. A statistical study of large numbers of larvæ revealed the proportions in which right-handed and left-handed individuals occur. Out of 916 specimens examined, 811 had the posterior cœlom on the left, 98 had it on the right, and 9 had it in a median position or so nearly so that no asymmetry could be made out. This means that over 88 per cent. exhibit left-handed asymmetry, over 10 per cent. right-handed asymmetry, and a little over 1 per cent. show no asymmetry. This is in contrast with conditions described by Gemmill (1915) for *Porania pulvillus*, where "a rudimentary enterocœlic outgrowth arises dorsally by proliferation of the stomach endoderm, usually on both sides of the median line, but sometimes in the median line, or on one side only. . . . When only one body is present it occurs rather oftener on the right than on the left side, a contrast to the condition in *Asterias rubens*, where the corresponding body (a still smaller one) appears most commonly on the left side." It seems evident from these observations that the presence among the asteroids of the posterior cœlomic vesicle and its asymmetry exhibits a highly variable incidence. The body is one that seems to be relatively vestigial in the group as a whole, but appears in a *Cribrella*, *Solaster*, and *Patiria* (doubtless also in other species) to be rather large and of some functional importance. It should also be noted that (in *Patiria*) the asymmetry exhibited by this vesicle always coincides with that exhibited by other cœlomic structures.

Development of Hydropores and Pore-canals.—On about the fifth day the larva gives off, typically from the posterior end of the left anterior cœlom, a dorsally directed hollow process, which comes into contact with the larval body wall and breaks through to form an open hydropore (Fig. 9). In *Patiria* the pore canal and hydropore constitute a large and conspicuous landmark for the determination of asymmetry. Without a single observed exception the hydropore is formed on the same side as the already formed posterior cœlom. There were a few cases in which the posterior cœlom appeared to be median, while the hydropore appeared only on one side. Attention should be called here to the fact that, while in *Poranus* (Gemmill, '15) the hydropore appears a day or so earlier than the posterior cœlom, exactly the opposite time relation prevails in *Patiria*, the posterior cœlom appearing a day or so earlier than the hydropore. Probably the earlier appearance of the posterior cœlom in *Patiria* accounts for its larger size and its permanency.

As was the case with the posterior cœlom, the hydropore also shows reversed asymmetry. The reversed or right-handed larvæ are as large, as vigorous, and as healthy as are the more typical individuals. A census of 652 individuals, belonging to seven different lots of larvæ, showed 589 with left-hand hydropores, 63 with right-hand hydropores, and none with both right-hand and left-hand hydropores; which means that over 90 per cent. are left-handed, and less than 10 per cent. are right-handed. We see then that the percentage of individuals with completed right hydropore is somewhat less than that of individuals with right posterior cœlom, but the difference is not significant, as both are very close to 10 per cent. Furthermore it may be said that the above censuses were made upon different lots of larvæ from those given for controls in the experimental part of the paper, and strongly tend to confirm the latter.

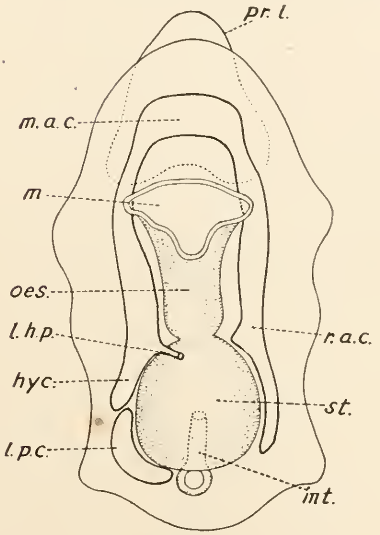
It should strongly be emphasized in this connection that the right-hand hydropores in *Patiria* are not merely temporary structures that subsequently regress and give place to left-hand hydropores, as Gemmill claims to be the case for *Asterias rubens*, but are permanent asymmetrical structures that remain up to the point of metamorphosis. In every culture observed the right-handed individuals maintained their reversed asymmetry and developed on the average as normally as did the more typical left-handed larvæ; in fact one gets the impression that in some cases at least, the reversed larvæ are a little larger and more active than the typical ones belonging to the same lot.

This is, I believe, the first time that large numbers of individuals with right-handed (reversed) asymmetry have been described for echinoderms. Only very rare and sporadic instances of reversal of asymmetry have been reported. In *Poranus*, a species of starfish studied intensively by Gemmill, double hydropores occurred in about 50 per cent. of the larvæ, but he specifically states that but "a single instance was observed in which the hydropore occurred on the right side alone." It may be pertinent, however, to recall that Oshima found both in control laboratory cultures and in experimental cultures of *Echinus miliaris* about 10 per cent. of specimens with right-hand asymmetry. In these experiments, however, it must not be forgotten that both controls and experiments were conducted under distinctly abnormal conditions, since artificial sea-water was used and the food was unsatisfactory. The possibility is not to be denied, however, that this species of echinoid may exhibit normally a fairly high degree of reversed asymmetry.

From what has been said, it may be inferred that *Patiria miniata* is in a somewhat delicate state of organic equilibrium with regard to its asymmetry, and should, therefore, furnish ideal material for an experimental analysis of the nature of this asymmetry.

The Development of the Hydrocœle and its Rays.—Although one may observe by the end of the first week or ten days a posterior prolongation of the left (in reversed larvæ, the right) anterior cœlom, this structure does not thicken up and become obviously a hydrocœle until about the end of the third week. In fact, a hydrocœle never develops unless the larvæ are well fed and otherwise well cared for. The hydrocœle appears at the same time and in the same manner in right-handed as in left-handed larvæ.

The Form of Bipennaria and of Brachiolaria Larvæ.—The form of the *Bipennaria* on the fifth day is shown in Fig. 9. It may be noted that the blastopore or larval anus has moved around to a distinctly ventral position; that the hydropore and pore-canal are well defined; that the posterior cœlom is now distinctly vesicular; and that both preoral and postoral ciliated bands have begun to be differentiated.



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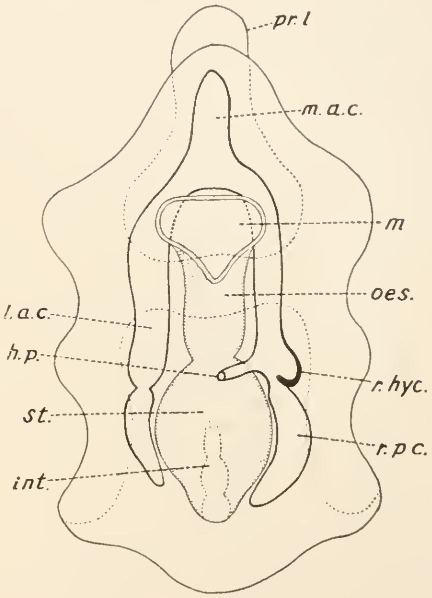

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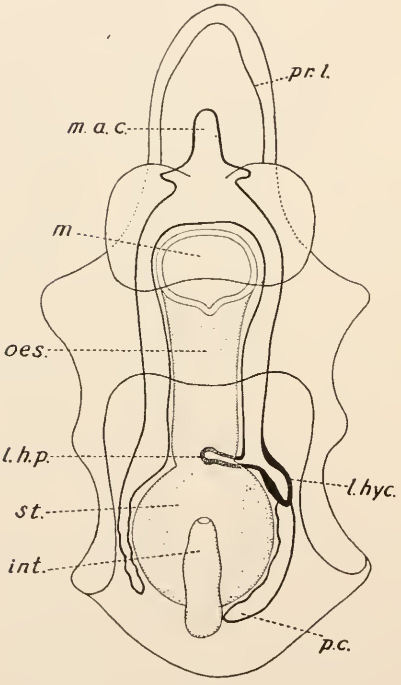
FIG. 10. Dorsal view of an advanced *Bipennaria* showing the ordinary left-handed asymmetry.

FIG. 11. A somewhat more advanced *Bipennaria* seen from the dorsal side and showing reversed or right-handed asymmetry. *h.p.*, hydropore; *hyc.*, hydrocœle; *int.*, intestine; *l.a.c.*, left anterior coelom; *l.h.p.*, left hydropore; *l.p.c.*, left posterior cœlom; *m*, mouth; *m.a.c.*, median anterior cœlom; *oes.*, œsophagus; *pr.l.*, preoral lobe; *r.a.c.*, right anterior cœlom; *r.hyc.*, right hydrocœle; *r.p.c.*, right posterior cœlom; *st.*, stomach.

At nine days the larva has undergone much change. The right and left anterior cœloms have grown forward beyond the mouth. The left (in reversed larvæ, the right) anterior cœlom has extended backward so as to be in contact with the posterior cœlom; while the right anterior cœlom has a posterior prolongation extending as far as the posterior end of the stomach. The preoral ciliated band is triangular and the posterior end of the postoral band is turned well forward toward the mouth.

At two weeks (Fig. 10) the anterior cœloms of the two sides have fused above the pharynx to form a continuous vesicle. The left anterior cœlom is still separate from the posterior cœlom, which is now quite large. The posterior prolongation of the right anterior cœlom has become relatively small in caliber, in fact has begun the process of rudimentation.

At twenty-four days (Fig. 11) the left anterior cœlom has



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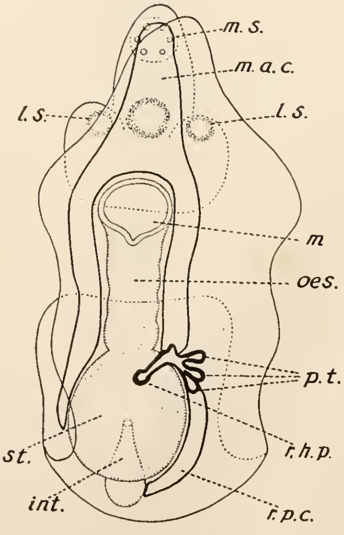

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FIG. 12. Advanced *Bipennaria* larva seen from the ventral surface, showing left hydrocœle well developed.

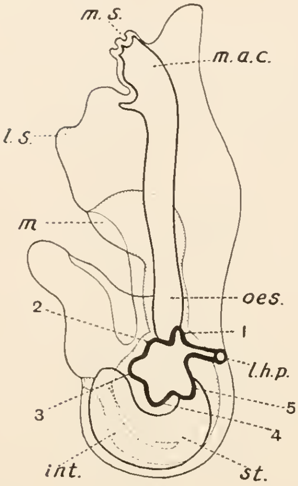
FIG. 13. Young *Brachiolaria* larva, seen from the dorsal surface, with reversed asymmetry. Primitive tentacles (*p.t.*) are shown arising from the hydrocœle of the right side. Right hydropore (*r.h.p.*) and right posterior cœlom (*r.p.c.*) The preoral lobe has become modified into the attachment organ of the simplified *Brachiolaria*, with median sucker (*m.s.*) and lateral suckers (*l.s.*). The remaining lettered structures are explained in previous legend.

united with the posterior cœlom; the hydrocœle has begun to undergo marked thickening of its walls; the median anterior part of the fused anterior cœloms has sent forward a long median process into the preoral lobe of the larva which has now a trilobed shape.

At twenty-six days (Fig. 12) the median diverticulum of the anterior cœloms has branched terminally into three parts, a large median one and two small lateral ones. The preoral lobe is now decidedly a trilobed affair.

At the end of a month the larva takes on the characteristic features of a simplified *Brachiolaria* (Fig. 13). The changes that signal the arrival of the larva at the Brachiolaria stage have to do with the differentiation of the median and the two lateral brachia of the preoral lobe, organs that were already foreshadowed in the trilobed form of the preoral lobe and in the three-branched condition of the median anterior cœlomic process. The middle brachium develops a well-defined attachment disk (Fig. 16) on which are present three pairs of papillæ. The lateral brachia remain simple knobs, slightly thickened and clubbed at the ends. One branch of the anterior extension of the cœlom runs into each brachium, but the lateral branches remain relatively small. A ventral sucker appears between the paired lateral brachia. The hydrocœle of the *Brachiolaria* has become lobed in some individuals, and in others it has given off some or all of the radial branches, destined to form the radial water canals (Figs. 13, 14, 15).

Further than this it was not possible to go with certainty. For one month the larvæ grew normally on a diet of the diatom, *Nitschia*, but almost over night the best cultures underwent retrogressive changes and died. One individual was found which had rounded up into a thin-walled vesicle. At first it was hoped that this individual had undergone or was undergoing metamorphosis, but in less than a day it also died. The same fate attended three other carefully reared lots of larvæ, all dying after about a month, and none, so far as I was able to determine with certainty, completing the process of metamorphosis. In spite of this ill fortune it should be emphasized that larvæ with right-handed (reversed) asymmetry were found among the most advanced of the *Brachiolariæ*, and would without doubt have developed the mouth and other oral structures of the adult from the right-hand side of the larva. If this type of symmetry reversal takes place sometimes in nature, as it doubtless does, there appears to be nothing about the anatomy of the radially symmetrical adult to indicate whether it has been derived from a larva with the normal or one with the reversed asymmetry.



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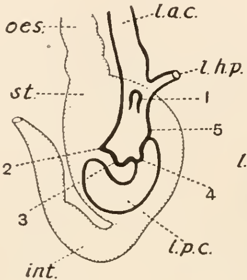

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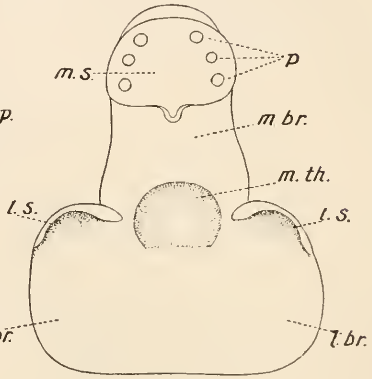

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FIG. 14. *Brachiolaria* larva seen from the left side. The primitive tentacles of the prospective adult are numbered 1, 2, 3, 4, 5. Other lettered structures the same as in previous legends.

FIG. 15. Posterior portion of another *Brachiolaria* larva, showing another stage in the formation of the prospective adult.

FIG. 16. Ventral external view of the preoral lobe of *Brachiolaria* shown in Fig. 14. The median brachium (*m.br.*) has a median sucker (*m.s.*) with six papillæ (*p*). There are two short lateral brachia (*l.b.*) each with a poorly developed lateral sucker (*l.s.*). Between the lateral suckers is a median thickening (*m.th.*) which may be another sucker.

In attempting to account for repeated failures to rear these asteroid larvæ through the period of metamorphosis, it has occurred to me that in nature there doubtless takes place a radical change in the dietary of the young individuals, which up to metamorphosis had been herbivorous, feeding largely upon diatoms, and that after metamorphosis they change to a carnivorous diet; for the adult is strictly carnivorous. Hence failure to metamorphose may be due to the absence of the forms of animal food essential for this process.

III. Experimental Induction of Reversed Asymmetry and of Bilaterality in *Patiria*.

Materials and Methods.

In all experiments dealt with in this paper the eggs of one female fertilized by the sperm of one male were used both for experimental and control lots. Large numbers of fully ripe eggs and sperm are readily obtained in the manner described in the introduction to this paper.

After several preliminary trials it was found that the blastula stage is the one best adapted for the experimental induction of reversals of asymmetry. Consequently all experiments herewith presented were performed upon early, middle, and later blastulæ, and early gastrulæ.

The experimental procedure was as follows: An ice box containing a large, flat layer of ice was the only unusual apparatus employed. A single lot of eggs was divided into two, three, or four equal lots, care being taken to avoid crowding. As a rule, a single layer of eggs on the bottom of the dish was found to be not too many to permit of normal development. One lot was always kept as a control, being placed from the time of fertilization on in an unheated room, resting upon a concrete table and shaded from too strong light. The experimental lots contained in covered glass vessels were placed directly upon the block of ice. After about half an hour temperature readings were taken and it was found that the water at the bottom of the dish where the embryos lay ranged from 2° to 3° C., this being about the temperature of the surface of the ice itself. At this temperature all progressive development comes to a complete standstill, but is resumed as soon as the normal growth temperatures are reinstated. In spite of the fact, which will be more fully dealt with in a subsequent paper, that development is inhibited at the experimental temperatures, the ciliated larvæ lived and remained active in some cases for as long as ten days, though kept continuously on ice for the entire period. This circumstance is mentioned here merely to emphasize the point that, though development ceases, certain phases of vital activity continue almost unabated.

In presenting the experimental results it seems advisable to give in some detail the results of the first complete experiment, which afterwards proved to be typical, and to follow this with a relatively condensed account of several nearly identical experiments that serve to corroborate the findings of the more intensively studied first experiment.

EXPERIMENT 1.

A fine batch of eggs, fertilized at 2.15 P.M., April 6, 1923, was divided into four equal lots. After 17 hours, when the embryos were still unhatched blastulæ, but were rolling about within the vitelline membrane, three lots were put on ice and one lot kept under normal conditions as a control. One lot (1, *A*) was kept on ice for one hour, a second lot (1, *B*) was iced for two hours, and a third lot (1, *C*) was iced for three hours. After exposure to low temperatures the experimental lots were placed beside the control lot in a room with temperature of about 15° C. and allowed to develop without disturbance for five days, at which time the first definite signs of asymmetry may be observed. On the fifth day both the control and the experimental lots were carefully looked over with reference to the percentage of larvæ showing reversal of asymmetry. It was obvious under casual observation that there were many more reversed larvæ in the experimental lots than in the control, but the exact difference could be determined only by means of a census of a representative number of individuals from each lot.

The plan adopted was that of random sampling. A few larvæ at a time were picked up in a pipette and transferred to a Syracuse watch glass and all examined to determine the position of the posterior cœlom, a rather conspicuous object at this time. When the count reached 100 the census was considered complete. This might be considered too small a number to give significant data, but when this is repeated a great many times it gains statistical value.

First Census (5 days old).

Control	87%	of larvæ have	left	posterior cœlom
	13%	" " "	right	" "
1, *A* (iced 1 hr.)	56%	" " "	left	" "
	44%	" " "	right	" "
1, *B* (iced 2 hrs.)	79%	" " "	left	" "
	21%	" " "	right	" "
1, *C* (iced 3 hrs.)	36%	" " "	left	" "
	14%	" " "	right	" "
	50%	" " "	median	" "

Note: In the last lot it was impossible to decide in just half of the larvæ whether the posterior cœlom was destined to go to the left or to the right. The culture was somewhat belated and at this time and the asymmetry matter was unsettled in many of the larvae. Yet those that have settled their asymmetry show 72 per cent. with left-hand asymmetry as compared with 28 per cent. with right-handed asymmetry, a result very closely consistent with that shown in the second census of the same lot.

Second Census (six days old).

Control	97%	of larvæ have	left	water pore and pore canal
	3%	" " "	right	" " " " "
1, *A* (iced 1 hr.)	71%	" " "	left	" " " " "
	29%	" " "	right	" " " " "
1, *B* (iced 2 hrs.)	74%	" " "	left	" " " " "
	26%	" " "	right	" " " " "
1, *C* (iced 3 hrs.)	72%	" " "	left	" " " " "
	13%	" " "	right	" " " " "
	15%	" " "	both right and left water pores and pore canals	

Third Census (ten days old).

Control	98%	of larvæ with	left	asymmetry
	2%	" " "	right	"
1, *A* (iced 1 hr.)	89%	" " "	left	"
	11%	" " "	right	"
1, *B* (iced 2 hrs.)	88%	" " "	left	"
	12%	" " "	right	"
1, *C* (iced 3 hrs.)	61%	" " "	left	"
	21%	" " "	right	"
	18%	" " "	bilateral symmetry	

Note 1.—At this time it was evident that the experimental larvae, especially those in Lot 1, *C*, were smaller and less advanced than the control. It was necessary to discard from those chosen for the census all distinctly abnormal larvæ and those in which no water pores or pore canals had developed, and there were many of these. The suppression of these structures in experimental lots is in itself an important result, and is considered simply a more marked expression of the same morphological response that is shown in disturbances of symmetry.

Note 2.—In connection with the 18 per cent. of bilateral larvae listed for Lot 1, *C* in the last census, it should be noted that the paired water pores and pore canals were rarely equal in size, sometimes that of the left side and sometimes that of the right side being larger.

Summary of Experiment 1.

1. There was a fairly high percentage of control larvæ in the first census showing reversed asymmetry of the posterior cœlom, but the numbers of control larvæ with reversed asymmetry in the later censuses was very small, only 3 per cent. and 2 per cent. respectively.

2. In every census (9 in all) the experimental cultures showed a much higher percentage of reversed larvæ than the controls. The average per cent. of reversed larvæ in experimental lots was 30.44, as compared with 4.66 for the controls. Thus there was over six times as high a percentage of symmetry reversal in experimental lots as in controls.

3. In Lot 1, *C* (iced 3 hours) there were many bilateral larvæ, only one of these appearing in any of the other lots.

4. The conclusion seems obvious that in this experiment the percentage of instances of symmetry reversal has been very markedly increased by retarding the development of the blastulæ. In other words, reversal of asymmetry and bilateral symmetry have both been induced experimentally.

5. To determine whether this experiment is exceptional or typical, eight other experiments were carried out using the same materials and methods, but with some variations in the stages at which the larvæ were iced and in the length of exposure to low temperatures.

EXPERIMENT 2.

Twenty-one hours after fertilization, when all larvæ were hatched and swimming about as late blastulæ and early gastrulæ, they were divided into three lots. One was left as a control, a second iced for one hour, and a third iced for two and one half hours.

First Census (5 days old).

Control	94%	of	larvæ	with	left	posterior	cœlom
	6%	"	"	"	right	"	"
2, *A* (iced 1 hr.)	81%	"	"	"	left	"	"
	6%	"	"	"	right	"	"
	13%	"	"	"	median	"	"
2, *B* (iced 2.5 hrs.)	63%	"	"	"	left	"	"
	31%	"	"	"	right	"	"
	6%	"	"	"	median	"	"

Second Census (One week old).

Control	92%	of	larvæ	with	left	water pore and pore canal
	8%	"	"	"	right	" " " " "
2, *A* (iced 1 hr.)	86%	"	"	"	left	" " " " "
	13%	"	"	"	right	" " " " "
	1%	"	"	"	bilateral	" " " " "
2, *B* (iced 2.5 hrs.)	76%	"	"	"	left	" " " " "
	24%	"	"	"	right	" " " " "

EXPERIMENT 3.

Twenty hours after fertilization, when all larvæ were free-swimming blastulæ or early gastrulæ, they were divided into three lots: one the control, one iced one and a quarter hours, and the third iced two and a quarter hours.

First Census (5 days old).

Control	84%	of	larvæ	with	left	posterior cœlom
	16%	"	"	"	right	" "
3, *A* (iced 1.25 hrs.)	69%	"	"	"	left	" "
	24%	"	"	"	right	" "
	7%	"	"	"	median	" "
3, *B* (iced 2.25 hrs.)	77%	"	"	"	left	" "
	19%	"	"	"	right	" "
	4%	"	"	"	median	" "

Note: There was one specimen in Lot 3, *B* (iced 2.25 hrs.), which had two full-sized posterior cœloms on the left side, one behind the other. This might be interpreted as incipient metamerism.

Second Census (one week old).

Control	87%	of	larvæ	with	left	asymmetry
	13%	"	"	"	right	"
3, *A* (iced 1.25 hrs.)	81%	"	"	"	left	"
	16%	"	"	"	right	"
	3%	"	"	"	bilateral	symmetry
3, *B* (iced 2.25 hrs.)	83%	"	"	"	left	asymmetry
	14%	"	"	"	right	"
	3%	"	"	"	bilateral	symmetry

Note: This experiment shows only a slight increase in the percentage of reversed asymmetry through icing. It should be noted, however, that a large percentage of the larvæ, though not quite so old as those in Experiment 2, were beginning gastrulation and thus past the period of maximum susceptibility to symmetry reversal. This will be shown more clearly in Experiments 8 and 9.

EXPERIMENTS 4, 5, 6, 7, 8, 9.

Six different lots of eggs from six different females, fertilized by the sperm of one male, were dealt with exactly alike except that some were allowed to develop a few hours longer before icing, and varying times of exposure to low temperatures were tried. Each culture was divided evenly into two lots, one for control and one for experiment. As all lots were allowed to develop for a week or more before the census, the results may be readily tabulated as though belonging to one census though it took about three days to complete the count.

Table I.

No. of Exper.	Stage at which Iced.	Time Iced.	Symmetry of Larvæ when Examined.
4.....	Early blastulæ	2 hrs.	Control—89% left asymmetry 11% right " Iced—76% left " 24% right "
5.....	Early blastulæ	3 hrs.	Control—83% left " 17% right " Iced—73% left " 27% right "
6.....	Advanced blastulæ	7 hrs.	Control—87% left " 13% right " Iced—72% left " 24% right " 4% bilateral symmetry
7.....	Advanced blastulæ	18 hrs.	Control—93% left asymmetry 7% right " Iced—69% left " 21% right " 10% bilateral symmetry
8.....	Early gastrulæ	1 hr.	Control—88% left asymmetry 12% right " Iced—87% left " 13% right "
9.....	Later gastrulæ	6 hrs.	Control—91% left " 9% right " Iced—93% left " 7% right "

Summary of Table I.

(*a*) In Experiments 4, 5, 6, and 7, in which blastulæ were used, the iced lots showed a marked increase of symmetry reversal over the controls. Including those showing bilaterality among the reversed types, there are over 27 per cent, on the average, of reversed larvæ, as compared with 12 per cent. for the controls.

(*b*) In Experiments 8 and 9, where gastrulæ were used, there was no significant increase or decrease in the percentage of larvæ with reversed asymmetry as the result of low temperatures. This is in accord with Experiment 3, in which a large proportion of the larvæ were gastrulæ and in which the increase in reversed larvæ was relatively slight.

(*c*) Only in Experiments 6 and 7, where the larvæ were exposed for long periods to low temperatures (7 and 18 hours, respectively) were any larvæ found with water pores and pore canals on both sides. It might then be said that bilaterality is harder to induce than reversal of asymmetry.

IV. Discussion of Experimental Results.

Patiria miniata is a species of starfish that either exhibits in nature a fairly high degree of reversed asymmetry or else is so susceptible to influences tending to affect the symmetry system that even control cultures cannot be reared without the induction of some degree of symmetry reversal. Three experiments were tried with the idea of determining to what extent symmetry reversal could be reduced by taking every conceivable precaution against conditions that might be subnormal. Using only the freshest eggs and a minimum sperm concentration, allowing only a few eggs to a dish, keeping the dishes in running sea-water, feeding the larvæ on a pure culture of the diatom, *Nitschia*, and introducing some fresh sea-water daily, there was found to be on the average about 8 per cent. of larvæ showing reversed asymmetry, which is not appreciably smaller than that seen in the controls in the various lots previously dealt with. One may conclude then that *Patiria* is a species in which left-handed asymmetry is not fully fixed, but that the symmetry system is in a somewhat plastic state, a state well adapted for experimental purposes.

Left-handed asymmetry is at the present time normal for echinoderms. Considerable work has been done which tends to prove that this asymmetry is present even in the unsegmented egg, and also that the first cleavage divides the egg into the prospective right- and left-hand sides. When twins are produced by the isolation of the blastomeres at the two-cell stage it has been found that one twin develops faster and becomes a more advanced larva at any given time than does the other. It seems then that one half of the egg, one of the two blastomeres, and one half of the larva is physiologically superior to the other in the sense that it has a higher general metabolic rate as expressed in more rapid growth. As a rule the superior side is the left side.

Studies of the development of double monsters, which are incompletely divided twins, have shown that, in these bilateral systems, whenever one component for any reason gains a physiological ascendancy over the other, there is a strong tendency for the superior component to remain normal and for the inferior individual to become subnormal and to be reduced to a condition of a mere parasite upon the normal component. Thus arises the condition known as that of the "autosite and parasite" type of double monsters. In extreme cases of this sort the "parasite" component is reduced to a rudimentary cyst embedded in the tissues of the parasite. This condition I have already interpreted as one of *dominance and subordination* in growth. According to this principle, which has been shown to hold good in large numbers of cases in both animals and plants, a rapidly growing or differentiating region, or apical region, in an organism tends to inhibit the growth or differentiation of like regions within the realm of its influence. This is true of equivalent bilateral regions, which may be conceived of as intense rivals. If one rival region gains an ascendancy over the other, the inferior region tends to be suppressed.

Applying this principle to echinoderm development, we can readily recognize the analogy to the "autosite and parasite" situation in double monsters. Commonly the left side acts as the superior or "autosite" component, and the right side as the inferior or "parasite" component. The result is that certain important and actively differentiating structures (the posterior cœlom, the hydropore, and the hydrocœle) appear first on the more rapidly developing left side, and that these structures suppress the development of equivalent structures on the right side and tend to reduce to vestiges certain other cœlomic structures of the right side that have already appeared. In other words, the typical adult echinoderm is morphologically the surviving left-hand component of a twin or double monster, and its radial symmetry is merely a sort of mechanical adjustment necessary in order to avoid too pronounced a one-sidedness.

If, however, the rate of growth of a larva be sharply checked in such a way that the physiological state of the right and left components are reduced to a parity, it should be possible to get an organism both halves of which are equivalent. As a matter of fact, this result is not infrequently realized in the bilateral larvæ that have arisen as the result of rather prolonged icing. The commoner result, however, is that the right side usurps the ascendancy typically belonging to the left side and gives rise to larvæ with reversed asymmetry. If the physiological condition of the two sides were really reduced to parity, we should always have, as the result of icing, bilateral larvæ; but we should not, a priori, expect a retarding agent to effect the two sides equally. On the contrary, the expectation would be that the superior side would be more seriously affected than the inferior, so that when recovery took place the originally inferior side would emerge from the growth retarding influence first and would then behave like a physiologically superior side throughout development. Two difficulties immediately present themselves. Why, it may be asked, are not all larvæ reversed? And why do not the right-handed larvæ, about ten per cent. of which seem to be present in each lot, reverse and become left-handed?

The second of these queries may best be answered first by admitting that there is no ground for denying that at least some of the right-handed individuals, under the conditions of the experiment, do reverse their already reversed asymmetry back to the specific left-handed asymmetry. If this be taken into account, we would have to add materially to the observed percentages of reversed larvæ, for a certain percentage of the left-handed larvæ that are counted as unreversed are doubtless reversed right-handers. But there is still a large percentage of larvæ that have failed to undergo symmetry reversal, and our problem is to account for their failure to reverse. The only solution of the problem is one that takes into consideration the well-known fact that no two eggs or embryos are alike in the degree of their susceptibility to inhibiting agents or in their responses to given agents. It is probable that only a limited number of larvæ at any one time are in just that state of susceptibility the normal response to which is symmetry reversal, and that these unsusceptible types are responsible for the surplus of left-handed larvæ in all experimental cultures. Other larvæ show other expressions of growth inhibition, while still other particularly hardy forms show no effects at all.

The problem that confronts us in this connection is in no essential respect different from the one that has faced experimental embryologists ever since the first eggs or embryos were subjected to conditions other than normal. It makes no difference whether one uses toxic chemicals, anæsthetics, low temperatures, or hybridization, the result is always the same: some individuals are affected in one way, some in another, and some not all. A lot of Fundulus eggs, for example, are exposed for a given time to a given intensity of X-rays. Some of the radiated individuals, as Miss Hinrichs has shown, develop into two-headed monsters, others become cyclopic, others show lesions of the heart or circulation, not to mention many other typical teratological conditions. Our present problem does not concern itself with any attempt to account for the individual differences in susceptibility among eggs of the same batch: this is a problem of heredity, not one of development.

To revert specifically to the present experiments, it should now be said that, although our attention has been focused upon symmetry reversal to the exclusion of any other effects of exposure to low temperatures, it would be unfortunate to leave the reader with the impression that everything else about the experimental larvæ was strictly normal. The truth is that some larvæ develop no cœlomic structures at all; that others show a tendency toward a metameric duplication of anterior or posterior cœloms; that occasionally a larva has a hydropore connected with the posterior cœlom as well as the anterior. It should also be added that in all experiments in which the icing of larvæ lasted more than about two hours, many other expressions of growth inhibition appeared, sometimes associated with symmetry reversal and sometimes not. My strong impression was that on the whole the larvæ with reversed asymmetry were rather more normal in other respects than those with typical asymmetry.

In conclusion then, it may be said that symmetry reversal, and that particular phase of it which we may call induced bilaterality, are merely some of the specific effects resulting from arresting the growth of the larvæ at the blastula stage. If the arrest takes place much earlier or much later than the blastula stage other expressions of differential inhibition prevail and there is no evidence of symmetry reversal. If eggs are iced during early cleavage or even before cleavage the particular response is *twinning*, while icing at other times give equally striking but different results. These matters, however, must be left for another study and another report.

One of the principal values accruing from experimental embryology is that it often throws light upon the mechanism of normal development. It would be strange, then, were we to fail to find in this study no suggestion as to a more complete understanding of the significance of asymmetry in the echinoderms and of that remarkable series of changes involved in metamorphosis. I venture, therefore, to conclude this paper with an attempt to reinterpret in physiological terms the phenomenon of echinoderm metamorphosis in general.

V. A Physiological Interpretation of Echinoderm Metamorphosis.

There are many remarkable ontogenetic transformations in nature, but none so radical as the metamorphosis of the echinoderm larva into the adult. In the metamorphosis of the caterpillar into the butterfly, the axes of polarity and of symmetry are carried over unchanged from larva to adult; in the metamorphosis of the tunicate tadpole larva into the adult sea-squirt there is a relative inhibition of the apical regions of both axes, yet the polarity and symmetry of the larva are maintained in the adult; but in the metamorphosis of the echinoderm both polarity and symmetry of the larva are, as it were, entirely ignored, and the axes of the adult are established practically *de novo* and without much reference to previously existing axiate relations.

In terms of the physiological gradients involved, it may be said that what apparently happens is, that the original gradients of the egg and of the larva become practically obliterated and a new major axis arises approximately at right angles to the larval major axis with its apical region at a point somewhere near the middle of the left side of the larval stomach at the point where the hydrocœle arises. The crucial event in this shifting of axiate relations is, morphologically speaking, the differentiation of the hydrocœle. This structure, as all know, arises as an outpouching of the posterior end of the left anterior cœlom (enterocœle). There comes a time in late larval life when all other growth changes cease and the enlargement and differentiation of the hydrocœle seem to be the main changes taking place. This rapidly growing region then assumes dominance over the rest of the organism, becomes the apical point of a new axis, and assumes control of the entire subsequent development. For some time after the new or adult axis has arisen the original larval axes are maintained in so far as the general external form and behavior of the individual are concerned, but sooner or later these yield to the increasing dominance of the new apical region, as may be seen by the casting off or resorption of the most characteristic features of the larva, such as the ciliated arms or bands, the preoral lobe with its mouth and accompanying structures, and in the rudimentation of the cœlomic structures of the right side.

The developmental history of the hydrocœle may briefly be reviewed. Physiologically, it represents a region of rapid cell proliferation in a larva which is, at the time in question, practically at a developmental standstill. As is well known, any rapidly growing region in an organism has the capacity of dominating the development of other regions about it and of suppressing the differentiation of similar or homologous regions elsewhere in the organism. It may also, in the course of time, inhibit the development of rival regions and cause their atrophy or resorption. Morphologically speaking, the events are as follows: The hydrocœle, at first a small lobe of the left anterior enterocœle, buds off five primary tentacles—destined to become the five radial water canals. These canals assume a radiate position about a central ring through which the new mouth passes. It is about this ring and its five—more in some species—primary tentacles that the new adult radial organization centers. The primary axis of the adult passes through the center of the water-vascular ring. This new axis can be called neither an antero-posterior axis nor an axis of symmetry; it is something quite *sui generis*, as is recognized by those who have given to the two poles the names "oral" and "aboral." The secondary axis is also quite unique, for there is no true symmetry, but merely a superficial semblance of radial symmetry.

Symmetry Relations of Larva and Adult.—From the standpoint of the original larval axes of symmetry, it is clear that the events that lead up to metamorphosis occur typically only on the left side. On this account the situation has long been thought of as an outstanding example of asymmetrical development, and there has been much speculation as to its morphological and its phylogenetic significance. Prominent among the speculations that have arisen in connection with this phenomenon are those that concern themselves with the phylogeny of the echinoderms. It is generally held that the ancestral echinoderm was, like the larva, bilaterally symmetrical and that hydrocœles and their derivatives occurred on both sides. The objection to this view is that such an organism would be biaxiate, with two mouths and oral surfaces, two water-vascular systems and nerve rings. In view of this serious objection, it would seem best to look upon the development of the new axis as an evolutionary process resulting from the establishment of a pronounced asymmetry, and that it was only in connection with this newly established asymmetry that the development of such structures as the new mouth, the nerve ring, the Aristotle's lantern in echinoids, and similar structures, takes place. This view does not preclude the possibility of an originally bilateral ancestor, but merely denies to such an ancestor the bilateral possession of such specialized structures as the hydrocœle and its derivatives that arise only in connection with the apical region of an entirely new axis.

When, however, larvæ are found with hydrocœles and kindred structures on both sides, evidence *seems* to be afforded for the theory that the ancestral echinoderm had two sets of hydrocœle structures bilaterally arranged. If such anomalous larvæ are to be considered as atavistic, how can we explain the occurrence of larvæ in which the hydrocœle and its appurtenances appear exclusively on the right side? Is the right-handed or reversed larva also an atavistic reminiscence of an ancestor that had right-handed asymmetry? Even the most obdurate phylogenist would hesitate to go so far; yet the occurrence of reversed asymmetry is commoner than is bilaterality, and both can be induced by the same experimental means. Moreover, the two conditions grade into each other; for there are many larvæ in which hydropores and such structures occur on both sides but in which one or the other side shows these structures in all states of partial rudimentation.

How then can we explain the occurrence both in nature and in experiments of reversed and bilateral larvæ? The previous parts of this paper have indicated that all anomalies of this kind are merely induced developmental disturbances and that neither bilateral nor reversed larvæ have any more phylogenetic significance than has the occurrence of occasional two-headed fishes or than the sporadic occurrence of sinistral asymmetry in a species of snail typically dextral.

The Nature and Origin of Asymmetrical Development.—The question naturally arises as to why one side of an echinoderm larva should develop structures not duplicated on the other. Two possible explanations suggest themselves: first, that the cells constituting one half of the larval body have a higher rate of metabolism and hence a somewhat more rapid rate of proliferation and of differentiation than those of the slower side; second, that the asymmetry is due to some environmental factor that influences one side only or the two sides differently. In support of the first explanation it should be said that the cells destined to form the two bilateral halves of the larva are set apart from a very early period. Driesch (1906) found that bilaterality was definitely established at the eight-cell stage, and presented data that tended to show that the first cleavage plane coincides with the sagittal plane of the larva. In a number of cases he found that when twin gastrulæ were formed from the two blastomeres these twins showed mirror-image symmetry. This observation I have been able to confirm in connection with studies of twinning in *Patiria* (Newman, '22). Bilateral symmetry then appears to be established at the beginning of cleavage and is probably predetermined in the unsegmented egg.

More significant still for our problem is the fact that, in a large proportion of twins produced from the physiologically isolated blastomeres of the two-cell stage, the rate of development of the two larvæ is markedly different. Almost invariably one of a pair of such twin blastulæ or gastrulæ within a common vitelline membrane is well in advance of the other. In an earlier stage it can be seen that one twin blastula has fewer cells and a smaller blastocœle than the other. In later stages one gastrula is relatively normal and quite active, while the other is not infrequently subnormal and shows signs of disintegration.

These data indicate that the asymmetry which is in later stages expressed in the unilateral appearance of the hydrocœle and its derivatives, is traceable to a very early stage of development and is probably present in the unsegmented egg. Moreover, it appears merely to consist of an inequality of metabolic rate between the antimeric halves of egg and embryo. The difference between the two sides is in no sense qualitative, as some writers prefer to believe, but is purely quantitative; for, as we have seen, all the structures that characterize the superior side may be inhibited on that side and may be thus allowed to appear on the other side, or they may appear on both sides at once in case the physiological state of the two sides is experimentally equalized. The basis of asymmetry in the typical echinoderm is then the result of a dynamic imbalance between the earliest primordia of the prospective antimeric halves of the embryo. On this account one side develops more rapidly than the other, especially in the region where the hydrocœle arises, and the differentiation of the hydrocœle and its derivatives tends to suppress the growth of equivalent structures on the opposite side. This view is consistent with the experimental work reported in this paper and with a large mass of morphogenetic work of other sorts. No claim of finality is made for the conclusions reached in this study, but it is hoped that some of the ideas expressed may form a starting point for future investigation.

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Vol. XLIX September, 1925 No. 3

BIOLOGICAL BULLETIN

THE FINDING OF THE QUEEN OF THE ARMY ANT *ECITON HAMATUM* FABRICIUS[1]

WILLIAM MORTON WHEELER.

After the peculiar, large-bodied, wingless females, or queens of the army ants of the American tropics belonging to the typical subgenus Eciton had been sought in vain for many years by many collectors I finally succeeded in July, 1920, in securing two queens of *Eciton burchelli* Westwood in British Guiana, and the same year published an account of their capture.[2] Since that time the queens of three other typical Ecitons have been discovered in rapid succession. In August, 1920, Mr. F. M. Gaige took the queen of *E. vagans* Roger in Colombia, but has not yet described the specimen. In 1923 Dr. Carlos Bruch published an account with fine figures, of a queen of *E. dulcius* Forel, subsp. *jujuensis* Forel, which he took in the Argentine.[3] He has also described a queen of *Eciton hetschkoi* Mayr, captured by Weiser in July 1923 in the same country.[4] The latter is not, however, a *true* Eciton, but belongs to the subgenus *Acamatus*, which occurs also in our Southern United States, from which I have described the queens of three species (*schmitti* Emery, *opacithorax* Emery and *carolinense* Emery). More recently Reichensperger has published a description of the queen of *E. quadriglume* Haliday, from four specimens taken by Franciscan brothers on the Rio Negro, Brazil in the fall of 1923.[5] This *Eciton* is very closely related to

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[1] Contributions from The Entomological Laboratory of the Bussey Institution, Harvard University, No. 244.

[2] "Observations on Army Ants in British Guiana," *Proc. Amer. Acad. Arts and Sci.*, 56, 1921, p. 291–328, 10 figs.

[3] "Estudios Mirmecológicos," *Rev. Mus. de la Plata*, 27, 1923, p. 172–179, 1 pl. 8 text-figs.

[4] "Descripción de la reina '*Eciton* (*Acamatus*) *Hetschkoi*' Mayr," *Rev. Soc. Argent. Cienc. Nat.*, 7, 1924, p. 232–235, 2 pls., 4 figs.

[5] "Das Weibchen von *Eciton quadriglume* Hal., einige neue ecitophile Histeriden und allgemeine Bemerkumgen," *Zool. Anzeig.*, 60, 1924, p. 201–213, 5 figs.

dulcius. Among the few remaining species of true *Eciton* of which the queen has persistently eluded capture is *E. hamatum.* During the past summer (1924) I was fortunate enough to secure a beautiful specimen of this insect from a bivouacking colony just behind the new tropical laboratory on Barro Colorado Island in the Panama Canal Zone. This find is, perhaps, the more important because *hamatum* is the type of the genus and because it has such a wide distribution (from Brazil to Mexico) and is so common that its raids have been noticed by nearly every zoological explorer in tropical America. It is, in fact, among the more than one hundred described species of *Eciton*, the one which has been longest known and most often cited in the literature since Fabricius first described the soldier in 1781.

At 8.00 A.M. on August 1st, after a heavy rain on the preceding day, while collecting behind the laboratory I encountered an army of *Eciton hamatum* foraging along the central trail (Fig. 1) and in the adjacent jungle. The workers were plundering numerous nests of ants (*Pheidole, Acromyrmex* and *Camponotus* species) and carrying away their helpless larvæ and pupæ. On one of the tall trees they had found a large nest of a yellowish wasp (*Polybia* sp.) and for some hours were bringing in the brood in great quantities. Dr. Curt Richter devoted the morning to watching the files, computing their rate of movement and the nature of their prey. By following the various converging columns we eventually located the colony which was bivouacking less than a hundred yards from the laboratory near the edge of the jungle. The great mass of ants presented an astonishing spectacle (Fig. 2). They had selected the base of a small tree, which, about 15 inches above the ground, was joined to the trunk of a young stilt palm by a looped liana and some twigs, together forming a horizontal frame. The larger tree trunk was inclined to the north so that the surface of the bark and the ground beneath were quite dry. In this spot, which had evidently been sheltered from the heavy rains for several days, the ants had congregated in a compact, cuboidal mass, 13 to 15 inches high and broad, and suspended from the frame above mentioned. Dozens of large workers hung by their claws from the twigs and supported solid clusters and curtain-like sheets of workers and soldiers, the

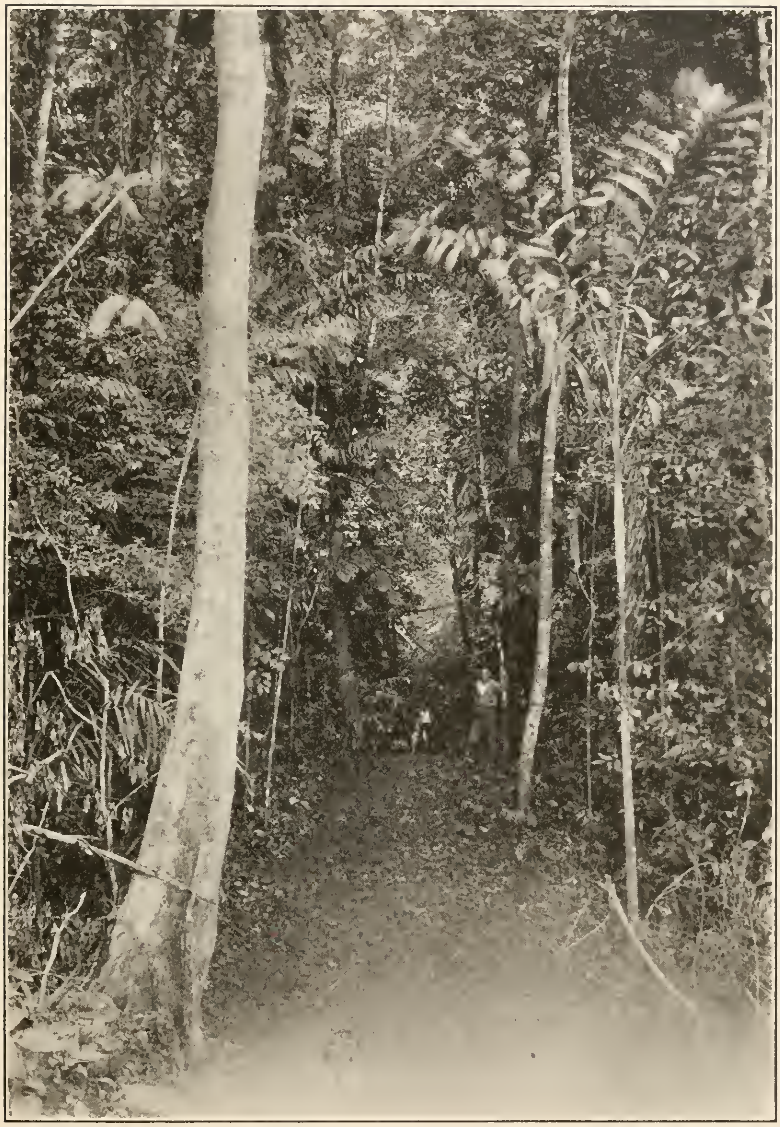

FIG. 1. Entrance to the central trail through the jungle on Barro Colorado. The Ecitons were foraging along this trail. Their suspended swarm (shown in Fig. 2) was situated in the jungle a few hundred feet to the left. Photograph by Dr. David Fairchild.

latter dotting the somber mass of intertwined brown-red bodies, legs and antennæ with their large shining, ivory-white heads. The mass hung down to the ground but left a small opening at the bottom on the west side, a kind of portal, through which the converging bootyladen files of workers and soldiers were continually pouring to deposit their burdens in the center of the mass and on the ground immediately beneath it.

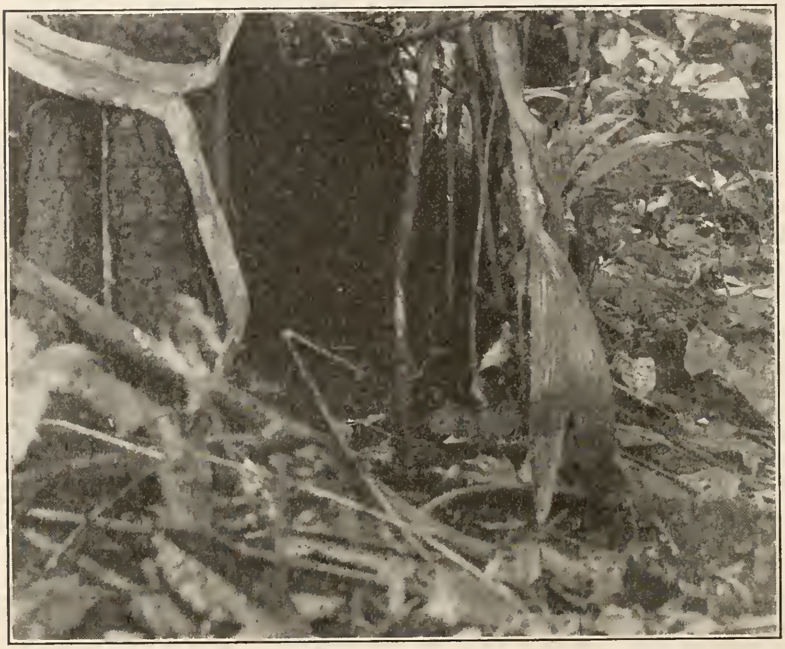

FIG. 2. Bivouacking colony of *Eciton hamatum*. The minute white dots are the heads of the soldiers scattered through the suspended mass of workers. Photograph by Dr. Frances G. Smith.

At my request, Miss F. G. Smith, who happened to be visiting the laboratory, kindly undertook to photograph the colony, and later Dr. Fairchild took a more enlarged flash-light picture. Considering the fact that the ants were clustered in a dark spot, the two photographs, which are shown in Figs. 2 and 3, give a good likeness of the shape of the cluster and of its composition. The blurred areas in Fig. 3 are, of course, due to the movements of several workers on its surface.

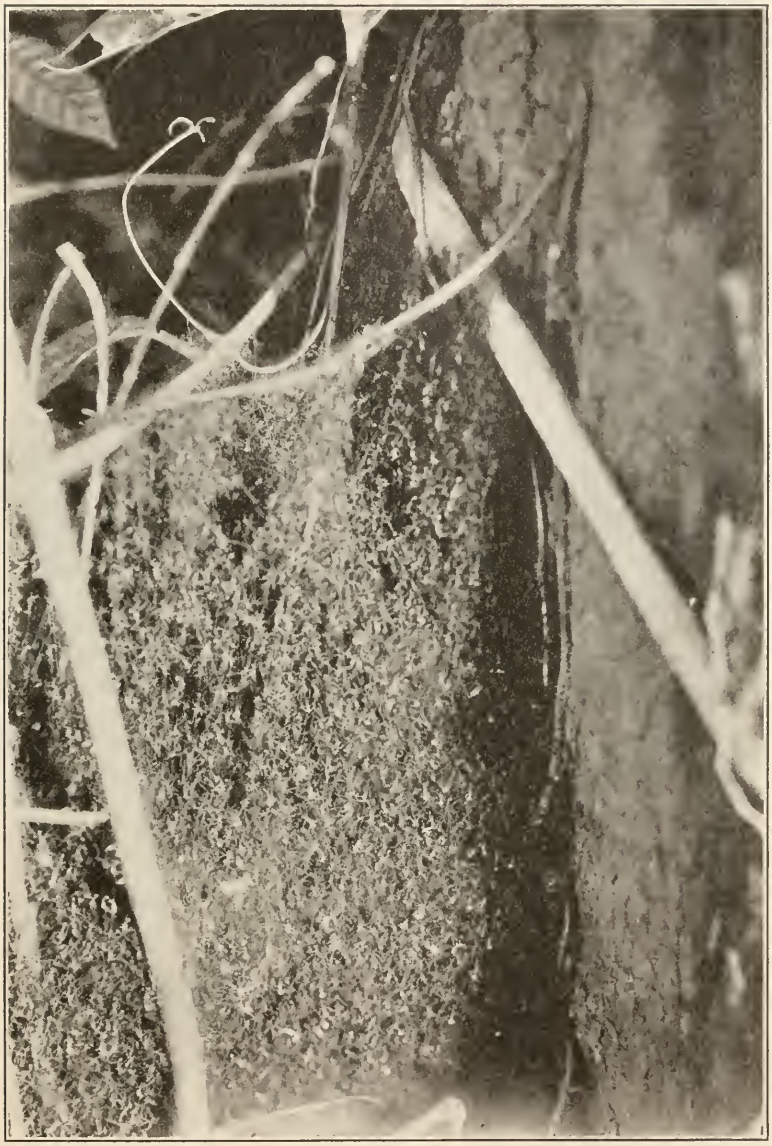

FIG. 3. Part of the suspended bivouacking colony of *Eciton hamatum* Fabr. on Barro Colorado Island. The interlacing legs and antennæ of the thousands of ants are distinctly shown. The white spots are the heads of the soldiers scattered among the swarm. About $\frac{1}{4}$ natural size. Photograph by Dr. David Fairchild.

Throughout the day the colony showed no essential change. It seemed very probable that the queen was concealed somewhere in the cluster, and although my enthusiastic young friend, Dr. Richter, wished to keep the colony under observation for several days, if possible, my arguments, inspired by fear that it might decamp during the night and disappear in the jungle and a desire to secure the unknown female and any guests, or ecitophiles that the mass might contain, eventually convinced him and my other companions, Mr. Nathan Banks, Dr. David Fairchild, his son Graham, and Mr. Frederick Burgess that it would be advisable to kill the whole mass and sort it over carefully at our leisure. The opportunity was, indeed, exceptional, because bivouacking colonies of army ants are rarely seen and when one is encountered it is almost always in some inaccessible place, in or under a large log, in a hollow tree-trunk or a hole in the ground. The destruction of the colony, however, seemed to be a serious matter, both because it involved eliminating one of possibly only a few

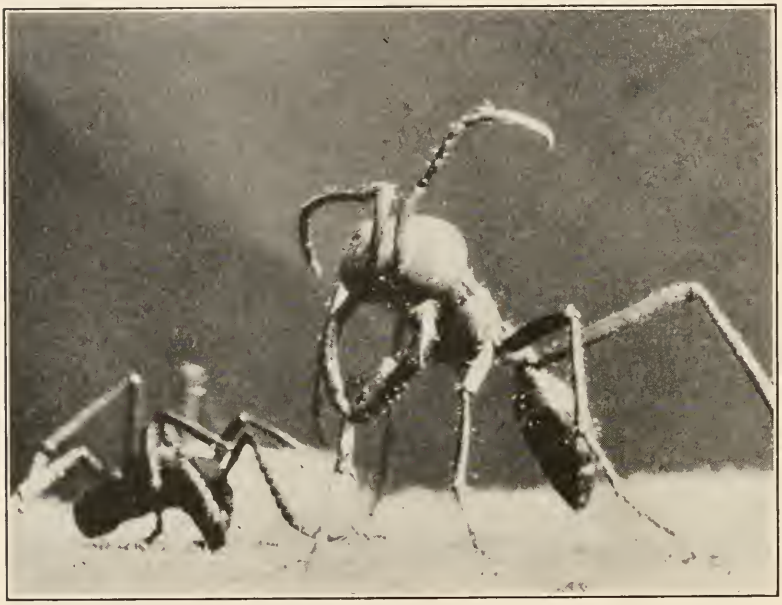

FIG. 4. Soldier and small worker of *Eciton hamatum* Fabr. Photograph by Dr. David Fairchild.

colonies of the species on the island, which is an animal and plant reservation, and because it is not an agreeable task to tackle a populous colony of one of the larger Ecitons. Fortunately *hamatum* is far from being as vicious or from stinging as badly as *E. burchelli*, though the soldiers (Fig. 4) are able to run their recurved mandibles into one's skin in such a manner as to make them as difficult to remove as an equal number of fish-hooks.

Just before dusk we matured and carried out the following plan: A towel saturated with ether was tightly wedged into the bottom of a five gallon gasoline tin from which the top had been removed. After cautiously cutting away the main supports of the framework of twigs, the tin was suddenly inverted over the mass of ants, and at the same time one of the party sprayed those left outside the receptacle with "Komo," a preparation used for killing house-flies and mosquitoes. The whole mass of asphyxiated insects, which measured two and one-half gallons, and comprised many thousands of individuals, was then examined. The queen was found, together with a great number of nearly full grown worker larvæ, but no pupæ, a considerable amount of prey, mainly ant and wasp larvæ and pupæ, and a series of interesting ecitophiles. On the following morning the *hamatum* workers and soldiers, which had been out foraging when the main body of their colony was captured, were found to have formed four small compact clusters, each about the size of an orange at widely separated points in the jungle. As usual under such circumstances these meager remnants of a vigorous population had a demoralized and dejected appearance. In the course of a few hours they decamped and disappeared in the undergrowth.

The *E. hamatum* queen (Figs. 5, 6 and 7) measures 15 mm. (head, thorax and petiole 6.5 mm., gaster 8.5 mm.) and differs from the queen of *E. burchelli* Westwood in the following particulars: The color is uniformly ferruginous red, of a distinctly paler tint than in *burchelli*, and there are no markings on the gaster, except some brownish clouds on the middle of the second to fourth segments, near their posterior borders, and a few brownish spots on the fifth segment. The mandibles are shorter and slightly broader, the antennal funiculi longer and stouter, the cheeks more inflated, the antennal foveæ larger and more sharply

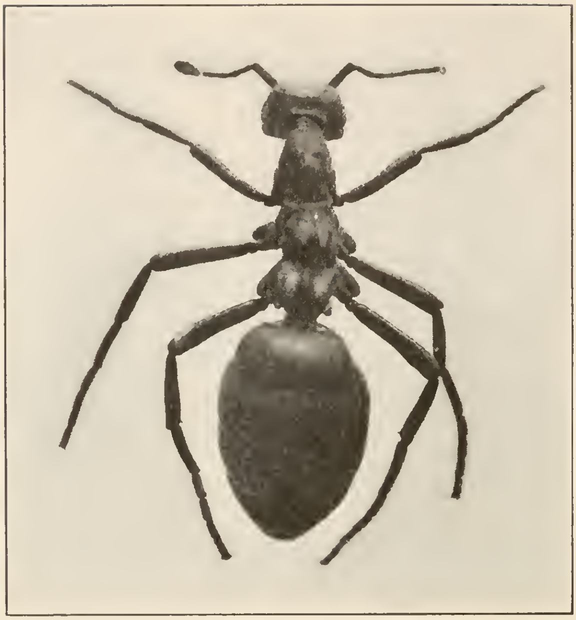

FIG. 5. *Eciton hamatum* Fabr. Female, dorsal view. Photograph by Prof. C. T. Brues.

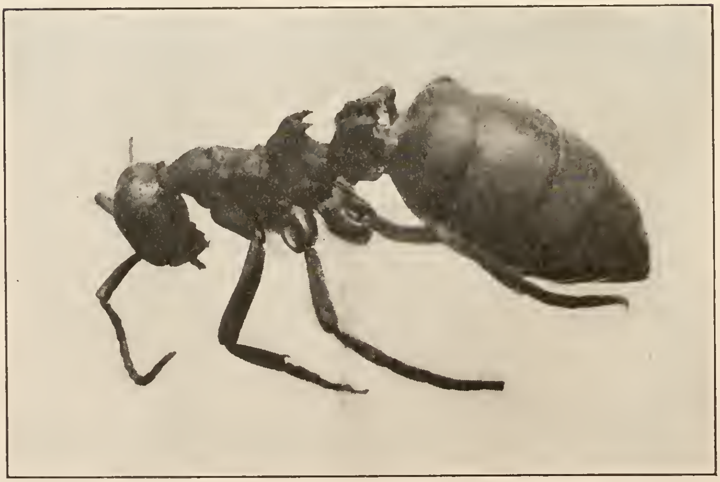

FIG. 6. *Eciton hamatum* Fabr. Female, dorsolateral view. Photograph by Prof. C. T. Brues.

defined and the eyes slightly larger than in *burchelli*. The pronotum is longer and more narrowed anteriorly, the mesonotum less convex and not distinctly grooved in the middle, the tubercles

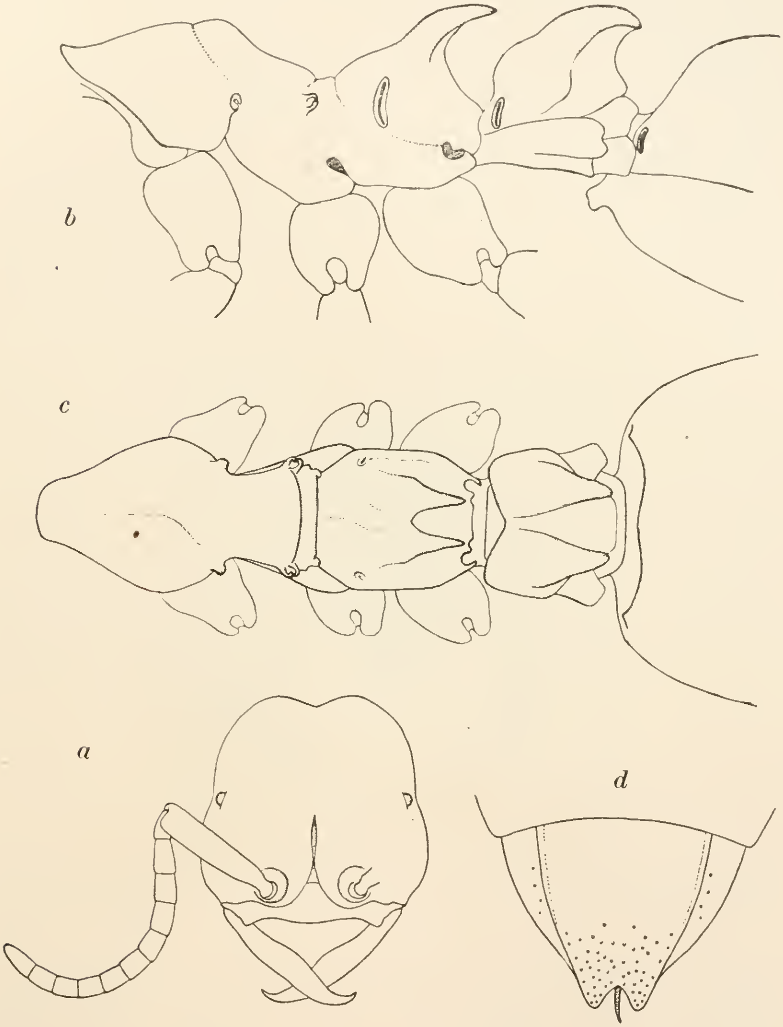

FIG. 7. *Eciton hamatum* Fabr. female; *a*, head, dorsal aspect; *b*, thorax and petiole in profile; *c*, same, dorsal aspect; *d*, hypopygium.

on the sides of the metanotum more pronounced. The horns on the epinotum are much longer and more pointed, those on the petiole much shorter, blunter and more rounded than in Westwood's species. The surface of the body, even of the sides of the epinotum and petiole are subopaque, the coarse punctures on the thoracic dorsum are much as in *burchelli*, but those on the convex dorsal portion of the first gastric segment are larger and more scattered. The hairs are shorter, finer, less golden and decidedly less abundant, especially on the legs, which in *burchelli* are very pilose. Other structural details may be gleaned from the accompanying figures (Figs. 5, 6 and 7).

This queen is obviously in the same physiological stage as the two queens of *E. burchelli* described in my former paper. Owing to the small size of her gaster she must be either a young individual or, if the mother of the numerous larvæ, workers and soldiers among which she was living, must have passed through a fecund period. The freshness of her color, integument and pilosity seems to support the former alternative. That the colony from which she was taken was not as large as some *hamatum* colonies I have seen, might also indicate that she was a young individual or one in which the gaster had temporarily returned to a contracted state after a first period (perhaps seasonal) of fecundity.

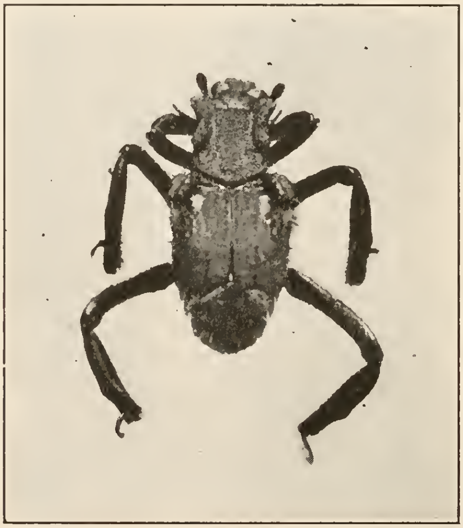

FIG. 8. Histerid ecitophile *Euxenister wheeleri* Mann, from bivouacking colony of *Eciton hamatum* Fabr. Photograph by Prof. C. T. Brues.

Of the eleven species of insects found in the bivouacking mass of *hamatum* workers and soldiers nine are Coleoptera, one a large Thysanuran (*Atelura* sp.) and one a mite resembling *Discopoma*. The beetles have been studied by Dr. W. M. Mann and Mr. H. S. Barber of the National Museum. The former writes me that there are five species of ecitophiles, namely two Staphylinids and three new species of Histeridæ. One of these is a *Troglosternus*, one a *Synodites* and the third, the remarkable form represented in Fig. 8, has been described by Dr. Mann as *Euxenister wheeleri*. The four remaining forms are bark-inhabiting or fungus beetles which were evidently brought in as prey by the foraging columns of ants. Mr. Barber identifies the specimens as a species of *Staphylinus* (*sensu lato*), two species of *Erchomus* and a *Rhymbus* (Endomychidæ). He sends the following note concerning the last, of which several specimens were taken: "The *Rhymbus* seems to be *Rh. hemisphœricus* Gerst. 1858, but not the species treated under that name by Gorham 1873 (Biol. C–A) although his *piceus* is perhaps a synonym of Gerstäckers species. Unfortunately an earlier generic and specific name (*Bystus coccinelloides* Guérin 1857, from Colombia) is listed under *Rhymbus* Gerst. 1858, and from its original description I cannot see why this latter name should not apply to the Barro Colorado specimens. The species is new to the National Collection."

A NEW GUEST-ANT AND OTHER NEW FORMICIDÆ FROM BARRO COLORADO ISLAND, PANAMA.[1]

WILLIAM MORTON WHEELER.

The researches of the past twenty-five years have shown that the number of ants which regularly live in more or less intimate symbiotic or parasitic relations with other ants is considerable, and that the behavior exhibited under these conditions is remarkably diverse. Until recently, however, such social parasites were known only from north temperate and subtropical regions. Several very interesting forms have now been discovered in the tropics and even in the south temperate zone. The following is a list of the workerless parasites (permanent social parasites) resembling the European *Anergates atratulus* Schenck and the North American *Epœcus pergandei* Emery that have been described from paleotropical and neotropical localities:

(1) *Wheeleriella wroughtoni* Forel (1910, 1911), described from female and male specimens found living in the nests of *Monomorium solomonis indicum* Forel at Poona, India.

(2) *Parapheidole oculata* Emery (1900, 1914–15), described from a female specimen from Madagascar and supposed to be a workerless parasite of some species of *Pheidole*.

(3) *Anergatides kohli* Wasmann (1915). Males and females taken in nests of *Pheidole megacephala melancholica* Santschi in the Belgian Congo.

(4) *Plagiolepis* (*Anoplolepis*) *nuptialis* Santschi (1917). Males taken in Cape Province by Dr. H. Brauns in nests of *P.* (*A.*) *custodiens* Sm.

(5) *Pseudoatta argentina* Gallardo (1916). Female and male described from specimens taken in Argentina and supposed to be parasitic in the nests of some fungus-growing ant of the genus Mœllerius, probably *M. balzani* Emery.

(6) *Xenometra monilicornis* Emery (1917, 1921), described

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 241.

from female specimens taken on the island of St. Thomas, W. I., in a nest of *Cardiocondyla emeryi* Forel.

(7) *Bruchomyrma acutidens* Santschi (1923), described from female specimens taken by Carlos Bruch in Argentina in the nest of *Pheidole strobeli richteri* Forel.

Besides the accounts of these extreme, workerless, permanent parasites numerous scattered and more or less incomplete notes have been published on other types of social parasites within the tropics. No slave-making ants have been recorded, but certain African and Malagasy species of Crematogaster of the subgenera *Oxygyne* and *Atopogyne* are probably temporary parasites in the nests of species of the typical subgenus Crematogaster, and the phenomenon known as "parabiosis," as Forel (1898), Mann (1912), and I (1913, 1921a) have shown, is well-developed among certain neotropical ants belonging to several genera (*Dolichoderus*, *Crematogaster*, *Odontomachus*, *Camponotus*). There is, moreover, in the tropics of both hemispheres a long series of tiny "thief," or lestobiotic ants, which belong to the Myrmicine genera *Solenopsis*, *Oligomyrmex*, *Aëromyrma*, *Pædalgus*, *Carebara*, *Erebomyrma*, *Tranopelta*, *Liomyrmex*, *Pheidole*, *Xenomyrmex*, *Monomorium*, etc. and live in or very near the nests of other ants or of termites.

During late July and early August, 1924, while studying the exuberant ant-fauna about the new tropical laboratory on Barro Colorado Island, in the Panama Canal Zone, I repeatedly came upon a small and peculiar *Megalomyrmex* living in the fungus gardens of a *Sericomyrmex*. Since the behavior of these insects represents a new type of symbiosis or xenobiosis, I here describe them, prefacing my account of each with a few historical notes. The taxonomic descriptions of the two ants and of some small lestobiotic species associated with the *Sericomyrmex* colonies are placed at the end of the paper.

Our knowledge of the habits of the Attine ants of the genus *Sericomyrmex* is rather meager. The earliest and best account is that of Urich published as early as 1895. It refers to a Trinidad species, later described as *S. urichi* Forel, but at the time of Urich's writing supposed to be *opacus* Mayr. "The nests of these ants," he says, "are found commonly about Port of Spain, in gardens, in the grass as a rule, but sometimes in the flower beds, and from their peculiar raised entrance can readily be recognized. They are always excavated in clayey soil, and the raised entrances, which are more or less cylindrical, are constructed with the particles of earth resulting from their mining operations and are about an inch in height. In young colonies this entrance leads into a small chamber, about six inches below the surface of the ground, situated, not at the end of the gallery but either to the left or right of it. As the colony increases the ants do not enlarge this original chamber, but, piercing its side, form another chamber near it with a small entrance hole. In large colonies, which never consist of more than about 200 individuals, a nest consists of two or three chambers which open on the original excavation. This is no longer used for growing the fungus in, but forms a sort of antechamber which generally contains material brought in by the ants to grow their mushrooms on, which is deposited here and gradually made use of. The chambers adjoining are more or less round, with a diameter of about 2–3 inches, and any small roots of plants growing through them are not cut away but used by the ants to hang their mushroom gardens on. These fill the interior of the chamber and consist of a gray spongy mass consisting of a great number of little irregular cells and resembling a coarse sponge, amongst which are scattered larvæ, pupæ, and ants. The walls of the cells consist of small round pellets resembling dust shot and are penetrated by and enveloped in white fungus hyphæ, which hold the mass together. Strewn thickly upon the surface of the garden are to be seen round white bodies about a quarter of a millimeter in diameter. These are what Möller terms "Kohlrabi" clumps, and consist of an aggregation of hyphæ with spherical swellings on their ends. It is on this that the ants feed. The fungus found by Möller in the nests of the Brazilian fungus growers (*Acromyrmex*) is the *Rozites gongylophora* Möller, and if it is not the same species cultivated by *S. opacus* it is, at any rate, very nearly related to it. As material to grow their mushrooms on the ants make use of particles of fruit, flowers, and leaves, but prefer the fruit. They do well in artificial nests and are easy to watch. I have tried them with all kinds of vegetable products; they have taken orange, banana, rose petals and leaves, and once they even made use of the dried glue from the back of an old book lying near their nest, but that day they had nothing else; if the choice be left to them they invariably take fruit and seem to prefer the orange among these. Very small particles of the white skin of the oranges are torn off, and, after undergoing a slight kneading process in the ants' mandibles, are placed in the nest. The neutres are all of the same size, varying but slightly and never exceed 4 mm. in length. They are more diurnal in their habits than other species of fungus growers, but also work a little at night. I have found winged forms in the nests in the month of July."

The following year Forel, while recording his observations on the Attini of Colombia, published the following remark (1896, p. 406): "The fungus gardens of the large *Atta* species, of the subgenera *Trachymyrmex* For. and *Mycocepurus* For., as well as of the genus *Sericomyrmex* were previously unknown and were discovered by me. The gardens of the three latter groups seem to resemble those of *Apterostigma*, and these small ants are never seen on the trees in the act of cutting leaves. They bring into their nests small, desiccated vegetable particles; their fungus garden lies very deep in the earth and is very imperfect." The Colombian species of *Sericomyrmex* (*S. diego* Forel) observed by Forel was not described till 1912 (p. 193). He then added the following note: "Don Diego, at the foot of the Sierra Madre de Santa Marta, Colombia, the third of March, 1896, in the forest; nest in the humus, with a crater of coarse granules. A beautiful fungus garden at a depth of 2 decimeters in the earth. The worker feigns death like the species of *Cyphomyrmex*. They collect little green vegetable particles resembling an alga and make their fungus garden of them and other débris." Essentially the same account was published by Forel in the "Biologia Centrali-Americana" (1899–1900, p. 37).

It will be noticed that Urich and not Forel was the first to observe the fungus gardens of *Sericomyrmex* and that the latter's various accounts contain some glaring discrepancies. In one account the garden is described as "very imperfect," in another as "beautiful." Furthermore, he could not have seen the fungus garden of *Mycocepurus*, which cultivates a peculiar fungus very similar to if not the same as the *Tyridiomyces formicarum* cultivated by *Cyphomyrmex rimosus* (Wheeler, 1907, p. 771).

For many years *Sericomyrmex* has been the one genus of Attine ants which I have not had an adequate opportunity to study. These insects are local or sporadic in their occurrence and very unobtrusive and timid in their behavior. Even on the few occasions when I have encountered them I could make but a superficial examination of their nests. My note-books contain only the following jottings:

FIG. 1. Laboratory of the Institute for Tropical Research on Barro Colorado Island, in Gatun Lake, Panama Canal Zone. Photograph by Mr. James Zetek.

Dec. 15, 1911, I happened on a number of nests of a small *Sericomyrmex*, which I have since described as *S. zacapanus* (1924) on the clay banks of a small irrigating ditch in an orchard at Zacapa, a very arid locality in Guatemala. The nests had small craters 2–3 inches in diameter, which were either single or in rows, like those of *Solenopsis geminata* nests, and were covered with the ejected fragments of exhausted fungus substratum. The soil was so hard that I could not reach the chambers and gardens which must have been some distance beneath the surface. The few workers that were abroad were bringing in small vegetable particles. During the summer of 1920 I examined some of the colonies of *S. urichi*, which were nesting in the lawn near Mr. Urich's laboratory in Port of Spain, but time to make a careful investigation was lacking. During the same summer I took in the sandy area adjoining the Tropical Laboratory of the New York Zoölogical Society at Kartabo, British Guiana, a few workers of a *Sericomyrmex* which I have recently described as *impexus* (1924), but I failed to reach the chambers in the very few nests that were excavated.

My sojourn on Barro Colorado Island finally yielded the desired opportunity to study not only *Sericomyrmex* but also several other Attini. During the height of the rainy season this locality is a veritable myrmecological and mycological paradise. Within a few hundred yards of the laboratory (Fig. 1) numerous colonies of at least 14 species of fungus-growing ants could be found, all with their gardens close to the surface of the ground and easily accessible. I recognized two species of *Atta*, one of *Acromyrmex*, three of *Trachymyrmex*, three of *Apterostigma*, three of *Cyphomyrmex*, one of *Myrmecocrypta* and one of *Sericomyrmex*. Thus nearly all the known genera of Attini were represented. Further search will probably reveal the presence of *Mycocepurus* on the island. Nor were the ants the only fungus-cultivating insects. The trunks of the trees that had been felled during the dry season (spring of 1924), when the small clearing was made around the laboratory, had reached a stage when they attracted thousands of ambrosia beetles of the family Platypodidæ. During June and July these insects were everywhere making their long tubular fungus-lined galleries in the dead wood and covering the logs with their frass. It would, indeed, be difficult to find a more favorable locality for mycological investigations, not only on account of the interesting fungi cultivated by so many ants and beetles but also of the extraordinary number and variety of other fungi, which during the rainy season flourish in all parts of the jungle.

Leaving an account of the other Attini for consideration at some future time, I will here confine my remarks to the *Sericomyrmex* which harbors the *Megalomyrmex* in its gardens. This Sericomyrmex seems to represent an undescribed species, which I shall call *amabilis*. It is very closely related to *S. impexus* Wheeler of British Guiana, but the worker is somewhat larger, the external borders of the mandibles are less convex, the mesothoracic tubercles are more acute and the silky hairs and pubescence, especially on the upper surface of the body, are longer and more conspicuous. The worker averages a little over 3 mm. in length, the female somewhat more than 5 mm., the male about 3 mm. All the phases are ferruginous brown, the female being decidedly darker and less reddish than the worker. Like most of the smaller Attini, the workers are very timid and inoffensive. When rudely touched they at once curl up and feign death. Owing to their gentle disposition and graceful and deliberate movements they are among the most fascinating ants to observe in artificial nests.

S. amabilis is probably common in many parts of the jungle on Barro Colorado Island but I was able to detect its nests only in the recently made clearing and trails, where the red clay, which gives its name to the island, is exposed to the sunlight, and there only some hours after one of the almost daily, heavy showers. As soon as the rain ceased the silky, mouse-like workers began to bring up small pellets of earth and carefully deposit them in the form of a loose crater around the entrance, which was about 3 mm. in diameter. These craters, which are completely obliterated by each rain, are probably large and noticeable during the dry season. As soon as one approaches the nest, the excavating workers, like those of *Trachymyrmex*, either feign death and become indistinguishable from the soil or hastily withdraw within the nest entrance, so that the observer must remain motionless for several minutes before they resume their labors.

Most of the nests observed had a single crater and entrance, with a slender gallery descending more or less obliquely to a chamber about 4 to 6 inches beneath the surface, but a few large nests resembled *Atta* nests, on a very diminutive scale in having several entrances and craters and as many as 3 to 5 chambers, scattered over an area of about a quarter of a square yard. The chambers varied in size from that of a pigeon's to that of a hen's egg, and were each filled with a fungus garden which was either entirely built up on the floor and walls or partly suspended from rootlets left intact by the ants during their excavations. The substratum of the gardens consisted of small, dull-yellow pellets of uniform size, held together and covered by a dense white mycelium, bearing minute clusters (bromatia) of spherical swellings ("kohlrabi") like those of *Atta*, *Acromyrmex* and *Trachymyrmex*. Urich's description of the gardens of *S. urichi* applies accurately to those of *amabilis*. The pellets of the substratum are undoubtedly particles of soft, chlorophylless vegetable matter collected by the workers in the immediate vicinity of the nest but I was unable to identify them more closely or to observe the ants in the act of gathering them.

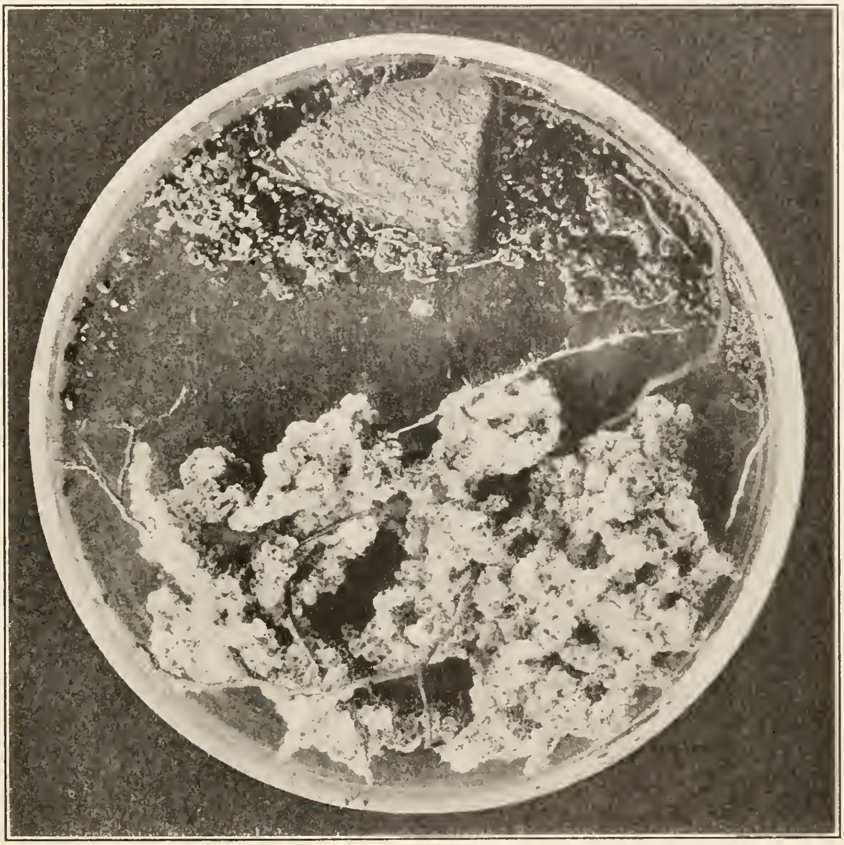

FIG. 2. Fungus garden of *Sericomyrmex amabilis* sp. nov. built in a Petri dish. × 1½. Photograph by Dr. David Fairchild.

In order to study the ants I placed them with their brood and fungus gardens in large Petri dishes. These made excellent artificial nests in which the insects could be kept in perfect health for two to three weeks and conveniently observed under a strong pocket-lens. Within 24 to 36 hours the frail fungus gardens, which inevitably fell to pieces when extracted from the earthen chambers, were completely reconstructed by the workers as an elaborate and rather regular sponge-work with polygonal crypts a quarter to half an inch in diameter. Of course, the flat space, less than half an inch in thickness, to which the ants were confined, compelled them to rebuild their garden in the form of a disc instead of a sphere or ovoid, but this was very advantageous, since it permitted the observer to scrutinize all parts of the structure through the glass cover. (See figures 2 and 3 from photographs by my friend Dr. David Fairchild.) The ants placed their eggs, larvæ and pupæ on the fungus-covered surfaces and in the crypts. The queen is a very sluggish insect and remained for long hours in a somnolent attitude near the center of the garden, or moved about very slowly and scattered her eggs in the immediate vicinity. These were rather large and broadly elliptical and were permitted to lie where they were laid till sometime after the larvæ had hatched. The workers then carried them to other parts of the garden and placed them in contact with fresh hyphæ. Since I never saw the workers administering hyphæ or "kohlrabi" to the larvæ as described for *Atta cephalotes* by Tanner (1892), I infer that the latter, when hungry, merely reach out and crop the fungus.

The larvæ are short, thickset and beset with sparse, long, flagellum-like hairs. The head is large and subrectangular, bearing small, acute mandibles covered with acute points. This type of mandible, which I find to be peculiar to the Attini, seems to be adapted to puncturing the delicate fungus hyphæ and expressing their juices. Worker pupæ were common in the nests, but pupal males and females were much less numerous. A few of the winged adult sexual forms emerged during the last week of July and the first ten days of August.

The workers were frequently observed in the act of building and rearranging the particles of the substratum of the garden and feeding on the "kohlrabi." They cropped the delicate hyphæ with their maxillæ, and not with their mandibles, without disturbing or shaking the substratum. On several occasions I saw

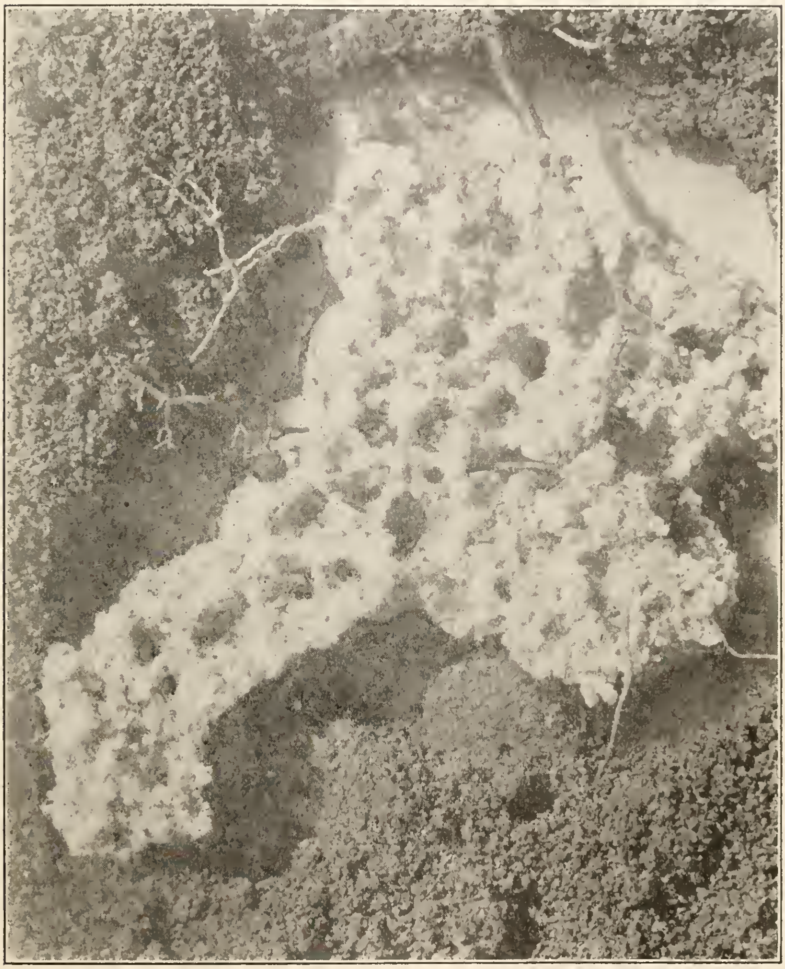

FIG. 3. Fungus garden of *Sericomyrmex amabilis* sp. nov. built in a Petri dish × 2. Photograph by Dr. David Fairchild.

them devouring injured larvæ or pupæ, and they greedily fed on the pulp of various fruits, such as mangos and bananas. They also tore off small bits of the inner rind of oranges and bananas and incorporated them in the garden. When such materials were not available, they manured the garden with numerous golden yellow droplets of their own feces. In two of my nests the gardens were suddenly blasted in a peculiar and unexpected manner. Bits of mango had been left in the Petri dishes and had decomposed during the night. This decomposition seemed to be due to some bacterium which was accidentally transferred to the gardens, probably on the feet and mouthparts of the ants, and at once overwhelmed the fungus, so that within a few hours it shrivelled up and turned black. The ants, apparently quite unable to prevent the inroads of the lethal microörganism or to restore their fungus to its normal condition, became demoralized and eventually wandered away from it to other parts of the dishes.

The population of the *Sericomyrmex* colonies which I examined, resembled that of *Trachymyrmex* colonies, the smaller nests containing between 100 and 200, the largest (a single nest) about 300 ants. No incipient colonies were seen, but there can be no doubt that the recently fecundated queens establish their colonies and gardens in the manner described by von Ihering, Huber, Gœldi and Bruch for various South American species of Atta and Acromyrmex.

The guest-ant, which I found in ten out of the twelve Sericomyrmex nests excavated in the immediate vicinity of the laboratory on Barro Colorado Island, is obviously a species of *Megalomyrmex*, a peculiar neotropical genus established by Forel in 1884 for a Colombian ant, *M. leoninus* and now known to comprise some 15 species which range from Bolivia to Mexico. While the generic name was appropriate to the type and several other species, which measure nearly a centimeter in length, it is a misnomer for several much smaller species—*gœldii* Forel, *pusillus* Forel, *wallacei* Mann, *silvestrii* Wheeler and *sjöstedti* Wheeler—which have been recently described. The known species of the genus are divisible into two groups, one of which, including the type, has convex, coarsely 5- or 6-toothed mandibles, with a sharp angle between their apical and basal borders, whereas in the other group, comprising only two species, *silvestrii* and *sjöstedti*, the mandibles are narrower and more flattened, with a rounded angle between the basal and apical borders and the latter with two large terminal teeth and a series of very minute basal denticles. This group is regarded by Mann as a distinct subgenus, for which he has proposed the name Wheelerimyrmex. I find that there is also a difference in the number of palpal joints in these two groups. In Megalomyrmex sens. str. (*M. bituberculatus* Fabr.) the maxillary palpi are 4-jointed, the labial palpi 3-jointed.[1] In *Wheelerimyrmex* I find the maxillary palpi to be 3-jointed, the labial palpi 2-jointed. The guest ant, which really represents a new subgenus and species and is described in the sequel as *Cepobroticus symmetochus*, has the same number of palpal joints as *Wheelerimyrmex*, but the mandibles are intermediate between the two other subgenera, having a sharp angle between the basal and apical border, and the latter with a large terminal and five or six small basal teeth. The antennæ, moreover, are short, all the funicular joints, except the last being decidedly broader than long and the clava absent. The promesonotal suture is obsolete but this character occurs also in one species of Wheelerimyrmex (*silvestrii*). In *sjöstedti* the suture is as distinct as it is in the species of Megalomyrmex *sens. str.* The eyes of the worker *Cepobroticus* are rather small.

It is, perhaps, significant that Emery (1921) has placed the genus *Megalomyrmex* in his tribe Monomorii, in the midst of a series of Old World genera—*Hagioxenus*, *Wheeleriella*, *Phacota*, *Xenomyrmex* and *Liomyrmex*—which are known to have xenobiotic or parasitic habits. Unfortunately very little is known concerning the habits of the described species of *Megalomyrmex*. The only data I have been able to secure are a few notes by Mann on *M. tuberculatus* and *M.* (*W.*) *silvestrii*. Of the former he says (1916, p. 445): "This form, which is confined to the upper Amazonian region, attends Membracidæ and the workers were generally found in company with these on shrubs in the dense forest. The nest is subterranean, the entrance nearly always at the base of a tree. The living insect is slow in its movements." Concerning *silvestrii*, which he observed in Honduras, he says: "A good series of workers were taken at Ceiba and San Juan Pueblo, nesting in the ground and in rotten logs. It is a timid species and very active when disturbed." These notes indicate that the species of Megalomyrmex *sens. str.* and the subgenus

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[1] Forel and Emery give 3 joints for each palpus.

Wheelerimyrmex are nonparasitic and epigæic. The occurrence of *Cepobroticus* only as a hypogæic inquiline, or guest in the fungus gardens of *Sericomyrmex* may be regarded as an ethological character which still further emphasizes its subgeneric status.

The *Cepobroticus symmetochus* worker measures only 3–3.5 mm., the female 3.8 mm., the male 3 mm. It is therefore the smallest known species of its genus. The body is very smooth and shining in all three phases and in the worker and female yellowish red, with the appendages scarcely paler and the dorsal median third of the gaster dark brown or blackish. The male is paler and more yellow throughout. All the castes, and especially the worker and female, are covered with rather coarse, long, golden yellow hairs. For other characters the taxonomic description and figures may be consulted.

The colonies of the *Cepobroticus* so frequently found living with *Sericomyrmex amabilis* were decidedly less populous than those of their host. The largest comprised less than 75 individuals, and often the number did not exceed 40 or 50. In every nest a deälated mother queen was present. She usually took up her station, surrounded by a group of her workers, in one of the crypts of the fungus garden a short distance—half to three quarters of an inch—from the *Sericomyrmex* queen. The guest ants kept their brood in small clusters scattered through the garden and each cluster was cared for by a few workers. Although the ants and their brood were thus intermingled, the workers of each species lavished their attention exclusively on their own eggs, larvæ and pupæ and were never seen even to transport the progeny of the other species from one part of the garden to another.

The workers and queens of *Cepobroticus* are rather alert and move about more rapidly than their hosts. They devote so much time to licking and fondling one another that the observer is somewhat astonished to find them paying little or no attention to the fungus-growers. As a rule the two species are indifferent to one another. One may watch them for hours without observing anything more than rather distant, mutual antennal salutations. On rare occasions a worker *Cepobroticus* may be seen licking the gaster of a *Sericomyrmex* worker or of the sluggish queen. More frequently one of the host workers may be observed in the act of lavishing similar but more elaborate attentions on a *Cepobroticus* worker. The fungus-grower begins by licking the feet or tarsi, the tibiæ and femora, then the thorax or abdomen and finally the head and even the mandibles of the guest. During this operation the latter remains motionless and inclines its body somewhat to one side.

The *Sericomyrmex* never feed their guests by regurgitation. This is not surprising because they never feed one another thus, but resort individually to the growing fungus bromatia. When hungry the *Cepobroticus* workers and queen also crop the fungus mycelium, but they do this rather roughly, using their mandibles and even shaking or disturbing the substratum. The guests very rarely transport or rearrange the particles of the substratum or take the slightest interest in the garden, except as a source of nourishment. Only on one occasion did I see a *Cepobroticus* carry a particle of the substratum to another spot, insert it and pat it down with her fore feet. When fresh fruit was introduced into the nest, it was much less frequently visited and eaten by the guests than by their hosts.

The larvæ and pupæ of the *Cepobroticus* can be readily distinguished from the *Sericomyrmex* brood. The larvæ are more slender and more cylindrical and have smaller heads, with flat, 3-toothed mandibles. The hairs on the body are more numerous, shorter and stouter, though rapidly tapering at their tips. I was unable to determine whether the larvæ are nourished by regurgitation or feed directly on the fungus hyphæ. The fact that they usually lie in the crypts in small clusters and in less intimate contact with the fungus than the *Sericomyrmex* larvæ would seem to indicate that they are fed by their nurses with regurgitated liquids.

The inquilines evidently lead a purely hypogæic life. Only the males and winged females leave the fungus chambers and come to the surface to mate. I took a few of the sexual forms which had thus escaped, and Prof. W. C. Allee, who collected on Barro Colorado Island during the spring of 1924, sent me among a number of miscellaneous ants a few winged females and several males of *Cepobroticus* which he had evidently taken on the ground or vegetation. The small size of the eyes in the worker as compared with other species of Megalomyrmex indicates that this hypogæic mode of life is beginning to affect the visual organs. Other obvious adaptive characters are the dentition of the mandibles, which is well suited to cropping the fungus hyphæ, and the investment of long, golden yellow hairs, which suggest a trichomal function like the golden tufts of many symphilic myrmecophiles.

Some experiments were conducted in mingling the personnel from different *Sericomyrmex* and *Cepobroticus* colonies. The former were so gentle and tolerant that when workers and queens belonging to different colonies were placed in the same Petri dish little animosity and that of very short duration was exhibited. Similarly, when inquilines from an alien colony were introduced, they were adopted at once without hostility, but the members of different colonies of the inquilines were much more hostile to one another. Frequently workers or queens would be dragged about for days and eventually mutilated or even killed by workers of their own species. This behavior was, perhaps, to be expected from what is known of the mutual animosity of parasites of the same species when confined with a single host.

The foregoing observations make it seem probable that the *Sericomyrmex-Cepobroticus* colonies are not established by a consociation of fecundated queens of the two species immediately after their nuptial flight, but that the *Cepobroticus* queen enters a well-established *Sericomyrmex* nest in which the fungus garden is already large and flourishing and being cultivated by a lot of workers. The development of the garden by the recently fecundated *Sericomyrmex* queen, as already suggested, evidently takes place in the same manner as in *Atta*, *Acromyrmex*, *Mœllerius*, *Apterostigma* and other Attini, and is such a slow and delicate operation that the presence of a fungus-devouring inquiline at the inception of colony formation would, to say the least, seriously interfere with the welfare of both queens. On the other hand, the intrusion of the *Cepobroticus* queen at a later stage, when the garden is well established, would not seriously affect the life and development of both colonies, especially as the inquiline is by no means a very fecund ant. This is shown by the small size of her own colony, her diminutive stature, and the small size of her gaster, which scarcely exceeds that of the worker. The fact that the workers show only a beginning in the reduction of the eyes would seem to indicate that the hypogæic and inquilinous habit is of rather recent phylogenetic origin. This supposition is also supported by the consideration that the Attini themselves constitute a young, or recent tribe of Myrmicine ants.

It is evident that *Cepobroticus* is merely a single aberrant species of *Megalomyrmex* which has abandoned an independent life, has associated itself permanently with *Sericomyrmex* and has taken to feeding on the fungus which it cultivates. The association thus established is a type of "compound nest," as defined by Wasmann, but differs from all the known types in certain important particulars. The relationship between the two species is somewhat like that obtaining between the xenobiotic *Leptothorax emersoni* and *Myrmica canadensis* in the mountains of our northern states and British America, but is in certain respects much less intimate. Although the *Cepobrotici* look after their own brood, they do not, like the *Leptothorax*, construct special chambers communicating with those of the host. Mutual feeding by regurgitation has not been developed, because both species feed on a delicate plant which is carefully provided and cultivated by one of them. We may, therefore, regard the relations of the *Cepobroticus* to the *Sericomyrmex* as a case of what the Germans call "Futterparasitismus," a case to which we might, perhaps, apply the term "mycetometochy." With the possible exception of the Pseudoatta described by Gallardo, we know of no other example of this relation among ants, but further investigation may reveal its occurrence among the termites of Africa and Southern Asia.

In the soil immediately surrounding the fungus chambers of some of the *Sericomyrmex* nests I found five minute species of ants which are described below as *Pheidole* (*Hendecapheidole*) *mendicula* sp. nov; *Oligomyrmex panamensis* sp. nov.; *Solenopsis conjurata* sp. nov.; *Tranopelta gilva* Mayr var. *columbica* Forel and *Rhizomyrma* sp. With the exception of the last these seem all to be "thief," or lestobiotic ants, but further observations will be required to establish their precise relations to the fungus growers. The most interesting species is the *Oligomyrmex*, because no representative of this genus, which is widely distributed over the warmer portions of the Old World—Southern Europe, Asia Minor, Africa, Madagascar, India, Indonesia, Papua, Australia—has been taken hitherto in any part of the New World. The new Hendecapheidole is also of interest, because only two species of the subgenus have been described, *tachigaliæ* Wheeler and *emersoni* Wheeler (1922), both from British Guiana.

TAXONOMIC DESCRIPTIONS.

Sericomyrmex amabilis sp. nov. (Fig. 4.)

Worker.—Length 3–3.5 mm.

Very close to *S. impexus* Wheeler but differing in its somewhat larger size and darker color and in the following structural details: The head is more deeply excised posteriorly, the eyes are distinctly larger and more convex, the posterior angles of the frontal lobes more acute, the continuations of the frontal carinæ which form the inner boundaries of the scrobe-like depressions for the antennæ, more pronounced, the mandibles with less convex external borders and more pronounced striæ. Their surfaces are at the same time more shining. The median joints of the antennal

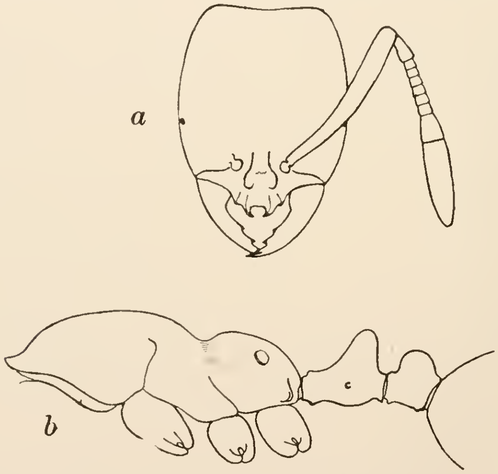

FIG. 4. *Sericomyrmex amabilis* sp. nov. *a*, head of worker, dorsal aspect; *b*, thorax and pedicel of same in profile.

funiculi are distinctly more transverse. The thorax, abdomen and legs are very similar to those of *impexus*, but the inferior angles of the pronotum, the pair of tubercles on this segment and the two pairs of tubercles on the mesonotum are somewhat larger and more acute and the lateral marginations of the gaster are more pronounced, as are also the three broad longitudinal depressions near its base on the dorsal side.

The pilosity and pubescence are decidedly more abundant than in *impexus*. The former is longer and more completely covers the integument, the latter is also longer and more conspicuous, especially on the dorsal surface of the body. The hairs are blackish at the base, with long, slender, flexuous, pale grayish or yellowish tips. The color of the body and appendages in mature specimens is rich ferruginous brown, with somewhat darker mandibles.

Female.—Length 5–5.5 mm.; wings 6.3 mm.

Similar to the worker. Backward extensions of the frontal carinæ and of the carinæ of the cheeks more acute, so that the antennal scrobes are more strongly developed. Mandibles coarsely striatopunctate. Pronotum with a blunt tubercle on each side, the inferior angles not very distinct. Mesonotum subrectangular, slightly longer than broad, flattened above, with a feeble Y-shaped impression. Scutellum less than twice as broad as long, bluntly bidentate behind. Epinotum declivous, with a pair of blunt longitudinal ridges terminating in blunt teeth. Petiole and postpetiole each with a pair of blunt longitudinal ridges above. The large first gastric segment is broader behind than in front, with straight sides, which are marginate; the three longitudinal impressions on the dorsal surface somewhat more distinct than in the worker.

Pilosity and especially the pubescence even longer than in the worker. Color darker, more brown and less reddish. Wings rather strongly and uniformly infuscated; veins and pterostigma pale, but very narrowly outlined with blackish.

Male.—Length nearly 3 mm.

Head, including the eyes, as long as broad, somewhat narrowed behind, with straight posterior border and rounded posterior corners. Eyes rather large and convex, the ocelli small and widely separated. Mandibles well-developed but narrow, their long apical borders finely denticulate. Antennæ slender, the scapes extending well beyond the posterior border of the head. Thorax large, the mesonotum convex anteriorly, with distinct Mayrian furrows. Scutellum trapezoidal, nearly as long as broad, feebly impressed in the middle, with entire posterior border. Petiole and postpetiole similar to those of the worker but the former more pedunculate anteriorly. Gaster small, oval; legs long and slender, the femora feebly bent.

Surface of the body smoother than in the worker and female, the mandibles and gaster somewhat shining, the remainder of the body subopaque.

Pilosity and pubescence very short and meager, only the sides of the petiole and postpetiole with tufts of hairs like those of the worker and female. Antennæ and legs destitute of hairs, with fine, indistinct pubescence.

Brownish yellow, head, a spot on the posterior portion of the mesonotum and a line on each side of it, brown. Wings colored as in the female.

Described from numerous workers, five females and a male taken on Barro Colorado Island, C. Z. during late July and early August.

This form is so close to *S. impexus* of British Guiana that it might be regarded as a subspecies. I have given it specific rank, however, because it now appears that there are several forms—*impexus* Wheeler, *urichi* Forel, *diego* Forel, *morierai* Santschi, *lutzi* Wheeler, *zacapanus* Wheeler, *opacus* Mayr, *pusillus* Forel and *aztecus* Forel—which are so closely related that they may be merely geographical races, or subspecies of one or a few highly variable species. At present our knowledge of these various forms and of their phases is so meager that it seems best to regard them as specifically distinct.

Megalomyrmex (*Cepobroticus* Subgen. nov) *symmetochus* sp. nov. (Fig. 5.)

Worker.—Length 3–3.5 mm.

Head subrectangular, very slightly narrower behind than in front, with straight posterior and very feebly convex lateral borders; the posterior corners rounded. Eyes small, feebly convex, at the middle of the sides. Minute ocelli sometimes present in large workers. Clypeus convex, its anterior border broadly and evenly rounded. Frontal carinæ short, parallel; frontal area indistinct, convex in the middle. Mandibles rather narrow and not very convex, 7–8 toothed, the apical tooth longer than the others which are subequal, the most basal forming the angle between the basal and apical borders. Maxillary palpi 3-jointed; labial palpi 2-jointed. Antennæ robust, the scapes extending less than one third their length beyond the posterior corners of the head; funiculi thickened apically, but not forming

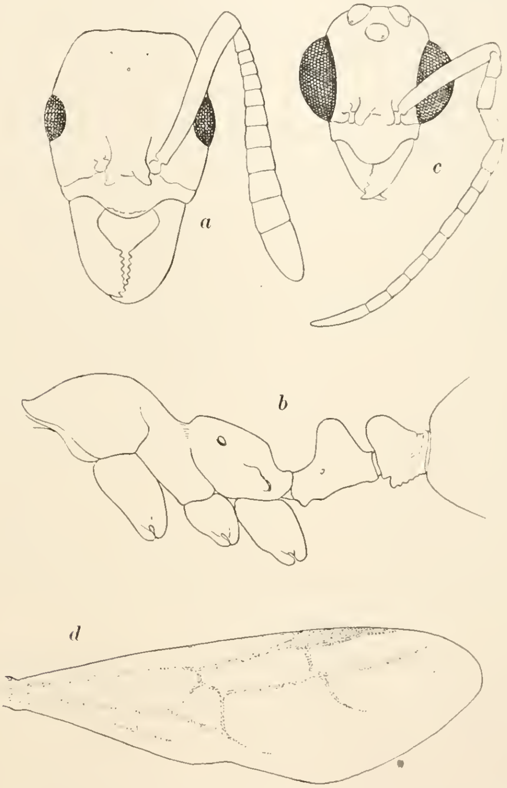

Fig. 5. *Megalomyrmex* (*Cepobroticus*) *symmetochus* sp. nov. *a*, head of worker, dorsal aspect; *b*, thorax and pedicel of same, lateral aspect; *c*, head of male, dorsal aspect; *d*, fore wing of female.

a distinct club; all the joints except the first and last broader than long; joints 2–4 transverse, nearly twice as broad as long. Thorax slender, narrower than the head; the pro- and mesonotum forming an even convexity above, without promesonotal suture; the mesoëpinotal impression distinct but shallow; the epinotum in profile rising steeply for a short distance in front, then becoming straight and horizontal in the middle and gradually passing into the short sloping declivity; the metasternal angles rather large, lamellate and rounded. Seen from above the epinotum has a ridge on each side, bounding a large median longitudinal impression for the accomodation of the petiole. The latter is nearly as high as long, the peduncle shorter than the node, which rises rather abruptly in front and is rounded above, with a posterior slope like the anterior. Seen from above the node is somewhat broader than long. The ventral surface of the peduncle bears a small, blunt tooth anteriorly. Postpetiole lower than the petiole and about half again as broad, with bluntly subangular sides, the node very convex above and inclined somewhat forward. There is a small acute tooth at the anterior end on the ventral side. Gaster elliptical, its anterior border feebly excavated. Legs rather slender.

Mandibles subopaque, densely striated; remainder of body very smooth and shining, with minute, sparse, piligerous punctures. Cheeks, mesopleuræ and sides of epinotum longitudinally rugulose.

Hairs long, erect or suberect, golden yellow, somewhat bristly and rather abundant on the body, legs and antennæ; pubescence absent, except on the funiculi.

Yellowish red; mandibles, funiculi, the posterior half of the first segment of the gaster and the sutures of the thorax and pedicel, brown; tip of gaster yellowish.

Female.—Length nearly 4 mm.

Very similar to the worker, with larger eyes and distinct ocelli. Thorax as broad as the head through the eyes, the mesonotum convex and rounded above, subhexagonal, as broad as long. Epinotum sloping, without distinct base and declivity. Gaster as in the worker. Wings with a distinct discoidal cell, a single elongate cubital cell and the submarginal cell open at the tip.

Sculpture, pilosity and color as in the worker. Each ocellus with a black margin internally. Wings yellowish hyaline, iridescent, with pale yellow veins and pterostigma; their membranes distinctly pubescent.

Male.—Length nearly 3 mm.

Head without the eyes longer than broad, with rounded posterior corners and somewhat convex posterior border. Eyes and ocelli very large. Anterior border of clypeus produced and rounded. Mandibles well-developed, with triangular denticulate blades. Antennæ slender; scapes nearly as long as in the worker; first funicular joint small, as broad as long, remaining joints, except the last, subequal, twice as long as broad, terminal joint somewhat longer. Thorax resembling that of the female. Petiolar node much lower than in the worker and female. Gaster elliptical, not excavated at the base. Legs very slender.

Sculpture very similar to that of the worker and female, but the sides of the thorax are smooth.

Pilosity also similar, but the wings with longer pubescence than in the female.

Brownish yellow, gaster a little darker, antennæ and legs slightly paler; eyes and a spot along the inner border of each ocellus black.

Described from numerous workers and females and two males taken from several colonies living in the fungus gardens of *Sericomyrmex amabilis* on Barro Colorado Island, C. Z.

I have made this ant the type of a new subgenus largely on account of the dentition of the mandibles and structure of the antennal funiculus. One unfamiliar with the smaller species of *Megalomyrmex*, especially those of the subgenus *Wheelerimyrmex* would be inclined to regard the new species as a *Monomorium*, mainly because the stature is so small, the mesoëpinotal constriction so feeble and the lateral ridges of the epinotum are so poorly developed as compared with other species of *Megalomyrmex*, but I believe that there can be no doubt concerning the natural affinities of the insect. Emery states (1921) that there is no discoidal cell in the fore wing of *Megalomyrmex*, but I have found it present in all the species I have examined. Apparently this cell may be either present or absent in the species of *Monomorium*.

Pheidole (*Hendecapheidole*) *mendicula* sp. nov. (Fig. 6.)

Soldier.—Length 1.3 mm.

Head very large, subrectangular, rather convex above, about $\frac{1}{5}$ longer than broad, as broad in front as behind, with nearly straight, subparallel sides and the posterior border rather deeply and semicircularly excised in the middle. The occipital and frontal grooves are rather shallow. Eyes small, convex, subtriangular. Mandibles not very convex, with rather straight external borders and two large apical teeth. Clypeus somewhat flattened, its anterior border straight and entire in the middle, sinuate on each side. Frontal area small and indistinct; frontal carinæ rapidly diverging, half as long as the head and forming sharp inner borders to rather deep scrobes for the accomodation of the antennæ, which are small. Scapes reaching to the middle of the sides of the head, the club as long as the remainder of the funiculus; joints 2–7 of the latter small and transverse. Thorax

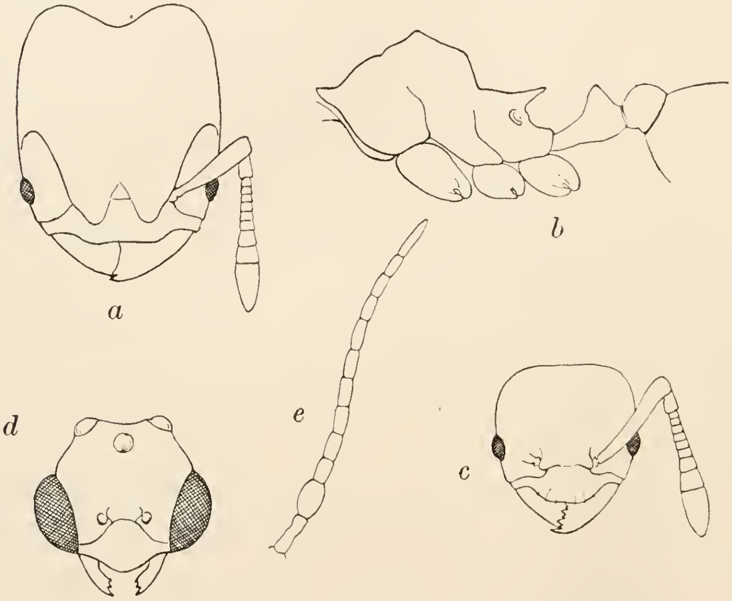

FIG. 6. *Pheidole* (*Hendecapheidole*) *mendicula* sp. nov. *a*, head of soldier, dorsal aspect; *b*, thorax and pedicel of same, in profile; *c*, head of worker, dorsal aspect; *d*, head of male; *e*, antenna of same.

short and robust, the pro- and mesonotum forming a mass which is very convex and subangulate above in profile, the posterior surface of the mesonotum descending perpendicularly to the pronounced mesoëpinotal constriction. From above the mesonotum is semicircular in front, with rather prominent humeri, behind which the sides are straight and converge to the mesoëpinotal constriction. Epinotum as long as broad, much lower than the promesonotum, with subequal base and declivity and two backwardly directed spines which are slightly longer than the width of their bases and nearly as long as the base of the epinotum. Petiole small, about 1½ times as long as broad, the peduncle distinct and parallel-sided, the node strongly compressed anteroposteriorly, with abrupt anterior and more sloping posterior surface and entire, distinctly transverse superior border. Postpetiole nearly half again as broad as the petiole; transversely elliptical, convex and rounded above and on the sides. Gaster smaller than the head, elongate-elliptical, with slightly concave anterior border. Femora and tibiæ distinctly thickened and clavate.

Shining; mandibles and clypeus smooth and very sparsely and finely punctate. Remainder of head densely punctate and transversely rugulose, the rugules most distinct on the front. Thorax and petiole densely and finely punctate, somewhat more coarsely on the promesonotum. Postpetiole and gaster very smooth and shining, the latter with a small punctate area near the insertion of the postpetiole.

Hairs yellowish, rather long and coarse, erect or suberect, moderately numerous, longest on the abdomen, sparser and somewhat shorter on the legs.

Brownish black, mandibles, sides of clypeus, antennæ and legs piceous; tarsi brownish yellow.

Worker.—Length 1 mm.

Head as broad as long, subrectangular, with distinct but rounded posterior corners, nearly straight posterior border and feebly convex sides. Mandibles with 7 small teeth, the second, fourth and sixth from the apex very minute. Clypeal border with four or five minute denticles which are the anterior terminations of longitudinal rugules. Eyes moderately convex, as long as their distance from the anterior border of the head. Frontal carinæ very short. There are no scrobes. Antennal scapes reaching to the posterior corners of the head. Thorax shaped much as in the soldier but the promesonotal mass is smaller and the humeral angles are less prominent, though the mesonotum is distinctly angular, with perpendicularly descending posterior surface. The epinotal spines are well developed but more erect than in the soldier. Postpetiole transversely rectangular, nearly 1½ times as broad as long and less than half again as broad as the petiole, which is similar to that of the soldier. Gaster truncated anteriorly. Legs as in the soldier.

In sculpture, pilosity and color very similar to the soldier, except that the head is merely densely and evenly punctate. The tips of the mandibles and the funiculi are paler and more brownish yellow in some specimens.

Male.—Length nearly 2 mm.

Slender; head, including the eyes, as long as broad, narrowed behind, with straight sides and concave posterior border. Eyes and ocelli large. Mandibles and clypeus small, the former tridentate, the latter convex in the middle, with rounded anterior border. Antennæ 12-jointed; scape very small and slender, scarcely longer than the swollen, ovoidal first funicular joint; joints 2–6 about twice as long as broad; 7–10 somewhat longer, the terminal joint slender and elongate. The funiculus tapers gradually to its tip. Thorax broader than the head, the mesonotum large, convex in front, as broad as long. Epinotum convex, with subequal base and declivity, rounding into each other. Petiole slender, parallel-sided, with very low and indistinct node; postpetiole somewhat broader, campanulate, as long as broad. Gaster and legs slender.

Shining; head subopaque and very finely and densely punctate; pronotum also finely punctate but more shining.

Pilosity yellowish, similar to that of the soldier and worker but shorter, especially on the legs, where the hairs are also more reclinate.

Yellowish brown; dorsal surface of body darker; head black; mandibles, mouthparts, antennæ, legs, insertions of wings and genitalia, pale yellow. Wings hyaline, with colorless veins and pterostigma.

Described from two soldiers, numerous workers and two males taken from a colony that was nesting in the soil immediately around the fungus chamber of a *Sericomyrmex amabilis* nest on Barro Colorado Island, C. Z.

This is quite distinct from the two other known species of *Hendecapheidole, tachigaliæ* Wheeler and *emersoni* Wheeler. The soldier of *mendicula* can be at once distinguished from that of *tachigaliæ* by its dark color and the very different sculpture of the head, the worker by its color and much stouter epinotal spines. The soldier *emersoni* is unknown, but the worker is paler than that of *mendicula*, much less pilose, with less developed epinotal spines. The male *emersoni* has a broader and differently shaped head, stouter petiole, coarser sculpture, darker wings and 11- instead of 12-jointed antennæ. The types of *tachigaliæ* were found inhabiting the petiolar swellings of a myrmecophyte (*Tachigalia paniculata* Aublet), those of *emersoni* a small cell within a termite nest (see Wheeler, 1921, p. 148, and 1922, p. 4).

Oligomyrmex panamensis sp. nov. (Fig. 7.)

Soldier.—Length 1.3 mm.

Head large, rather flat, fully $1\frac{1}{3}$ times as long as broad, very slightly broader in front than behind, with straight, subparallel sides and deeply, semicircularly excised posterior border. A well-developed anterior ocellus is present. Eyes very small, situated about $\frac{1}{3}$ the distance from the anterior to the posterior corners of the head. In the specimen the right eye is larger and pigmented, the left very minute and colorless. Mandibles short and convex, with about five blunt teeth. Clypeus very short and abrupt, its anterior border bluntly bidentate, sinuately emarginate in the middle and on the sides. Frontal carinæ short but well-developed, rapidly diverging. Antennæ small and slender, 9-jointed; the scapes reaching the lateral border of the head at points two fifths the distance from its anterior to its posterior corners. The 2-jointed club is as long as the remainder of the funiculus, the terminal joint large and swollen, fully three times as long as the penultimate, which is distinctly longer than broad; joints 2–4 subequal, broader than long; 5–6 nearly as long as broad, the basal joint as long as 2–5 together. Thorax narrower than the head, elongate, broadest through the pronotum, which in profile is rounded in front and straight and horizontal behind, where its outline is continued into the straight, horizontal outline of the mesonotum. There is no mesoepinotal constriction but there are very small though distinct scutellar and metanotal

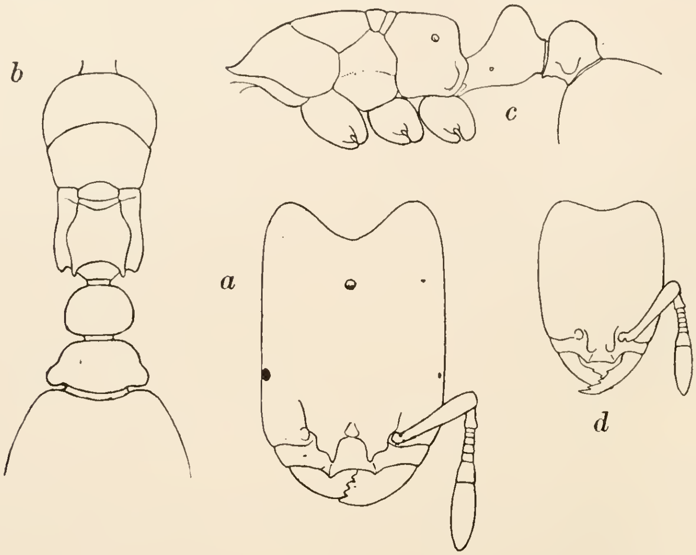

FIG. 7. *Oligomyrmex panamensis* sp. nov. *a*, head of soldier (or ergatoid?), dorsal aspect; *b*, thorax and pedicel of same, dorsal aspect; *c*, same, in profile; *d*, head of worker, dorsal aspect.

sclerites. The epinotum is subrectangular in profile, with an abrupt declivity shorter than the straight horizontal base. The angle on each side is formed by a ridge which is most distinct on the declivity. The surface between the two ridges is slightly concave. Petiole with a very short peduncle, which bears a strong, forwardly directed anteroventral tooth. The node is large and rounded, with rather steep, straight anterior and more abrupt, rounded posterior slope; from above it is transversely elliptical and nearly as long as broad. Postpetiole lower than the petiole, convex above, with a large, rounded tubercle on each side below and a minute anteroventral denticle. From above this segment is nearly $1\frac{1}{3}$ times as broad as the petiolar node and of a similar shape. Gaster about the size of the head, elliptical, somewhat flattened, its basal border rather straight. Legs short and slender.

Mandibles somewhat shining, finely punctate. Head subopaque, densely, finely and evenly longitudinally rugulose, the rugules straight and feebly diverging from between the frontal carinæ to the posterior corners. Thorax, petiole and postpetiole also subopaque but the pro- and mesonotum, and especially the scutellum, more shining; the pronotum indistinctly and very finely longitudinally striate. Gaster shining, with rather numerous, minute, piligerous punctures.

Hairs yellowish, suberect, sparse, more numerous on the gaster, fine and subappressed on the appendages.

Ferruginous red; legs, funiculi, except the articulations, and gaster paler and more yellowish; borders of mandibles and clypeus brown or blackish.

Worker.—Length 0.9 mm.

Head shaped somewhat as in the soldier, but much smaller, with evenly convex sides and more feebly excised posterior border. Eyes and ocelli absent. Mandibles less convex, with oblique blades, bearing three large apical teeth and a small basal tooth. Clypeus resembling that of the soldier. Frontal carinæ very short. Antennæ 9-jointed, the scapes reaching to the middle of the sides of the head; the terminal joint of the club proportionally longer than in the soldier, joints 2–6 of the funiculus decidedly shorter and more transverse, fully twice as broad as long. The thorax lacks the scutellar and metanotal sclerites and has a small but distinct mesoëpinotal constriction. Epinotum small, with subequal base and declivity, the former slightly convex, the latter sloping, the angle between the two obtuse and rounded. Petiolar and postpetiolar nodes subequal, the latter nearly as long as broad, rounded on the sides, without tubercles. Gaster much smaller than the head, with somewhat concave anterior border.

Smooth and shining, with scattered piligerous punctures, which are most distinct on the head, especially on its sides.

Pilosity much as in the soldier but the hairs are decidedly shorter and of more uniform length. Clypeus with four stout bristles. Hairs on the front directed transversely, on the sides of the head forward.

Yellow; legs and antennal funiculi somewhat paler.

Described from single soldier and worker specimens found in the soil surrounding a fungus chamber of *Sericomyrmex amabilis* on Barro Colorado Island, C. Z.

This minute ant, the first *Oligomyrmex* to come to light in the New World, closely resembles its Old World cousins, except in the shape of the thorax in the soldier. The fact that the eyes on the two sides of the head are differently developed indicates that it is somewhat abnormal, and since the thorax is somewhat like that of a female in possessing scutellar and metanotal sclerites the specimen may prove to be an incomplete ergatoid or pseudogyne.

Tranopelta gilva Mayr var. *columbica* Forel.

A small colony of workers with larvæ of what I take to be this form, originally described as a variety of *T. heyeri* Forel, was found in the earth immediately surrounding a fungus-chamber of *Sericomyrmex amabilis* on Barro Colorado Island. It is obviously very close to the var. *albida* Mann of Matto Grosso, Brazil, but the eyes are even smaller. The mesoëpinotal impression is a trifle less pronounced, the color is whitish as in *albida* and the pilosity is the same. It is interesting to note that Forel found the types of *columbica* at the bottom of the nest of a fungus-grower *Mycocepurus smithi* Forel. Another colony, however, was taken by him "in a subterranean nest, beneath dried cow-dung."

Solenopsis conjurata sp. nov. (Fig. 8.)

Worker.—Length 1.4–1.5 mm.

Head subrectangular, distinctly longer than broad, with feebly convex sides and slightly concave posterior border. Eyes minute, consisting of 5 or 6 abortive but pigmented ommatidia, placed one-third the distance from the anterior to the posterior corners of the head. Mandibles narrow, with oblique 4-toothed apical borders. Clypeus with the two median teeth stout, acuminate and turned inward, the lateral teeth short, broad and blunt. Antennæ rather slender; scapes reaching the posterior fifth of the head; basal funicular joint as long as the three succeeding joints together; joints 2–7 subequal, distinctly broader than long; the 2-jointed club somewhat longer than the remainder of the funiculus; the terminal joint fully three times as long as the penultimate, which is distinctly longer than broad. Thorax rather slender, the promesonotum longer than broad, somewhat depressed above, its outline in profile rather straight in the middle; mesoëpinotal constriction distinct but not very deep; epinotum small, as long as broad, in profile convex, rounded and sloping, without distinct base and declivity. Petiole small, the peduncle short, with a blunt anteroventral tooth, the node rather conical, rounded, as long as broad when seen from above. Postpetiole globular, a little broader than the petiolar node, somewhat broader than long. Gaster as large as the head, elliptical, with straight anterior border. Legs rather slender.

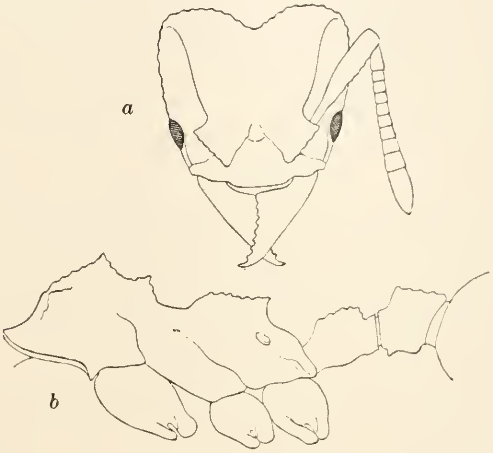

FIG. 8. *Solenopsis conjurata* sp. nov. *a*, head of worker, dorsal aspect; *b*, thorax and pedicel of same in profile.

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Smooth and shining throughout, with very fine, sparse, piligerous punctures.

Pilosity whitish, moderately long and abundant, erect on the body, more reclinate on the legs and scapes.

Uniformly pale yellow throughout, only the teeth of the mandibles reddish and the minute eyes black.

Described from numerous specimens belonging to a populous colony which was living in the earth surrounding a fungus chamber of *Sericomyrmex amabilis* on Barro Colorado Island, C. Z. These minute ants were kept for several days in an artificial nest with the Sericomyrmex and their guest ants but though they mingled freely with the large ants remained quite unnoticed. It would seem therefore that *S. conjurata* may be a true thief-ant like many other species of the genus (*S. molesta* Say, *fugax* Latr., etc.).

I have described this Panamanian ant as new because it does not agree with any of the neotropical species of which I have seen specimens or descriptions. In Emery's key it runs down to *S. helena* Emery, but this species, judging from his figures, has a much more rectangular head and very different teeth on the clypeus.

Rhizomyrma sp.

A single pale yellow, deälated female, clearly referable to this difficult genus and measuring only 2.3 mm. was found in the same situation as the preceding species. It is very probably undescribed but it seems best not to give it a name till the cospecific worker comes to light.

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PALM AND SOLE STUDIES.

VIII. Occurrence of Primitive Patterns (Whorls).[1]

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Introduction.

If we are correct in our morphological interpretation of the friction-skin patterns of the human palms and soles (Miss Whipple, 1904; H. H. Wilder, 1916) considering them the vestiges of the former walking-pads, we should expect to find upon each of these surfaces the typical set of eleven patterns, each in its proper topographical position. These are, in either hand or foot,

Five Apical or *terminal*, upon the balls of the terminal phalanges of the five digits, fingers or toes.

Four Interdigital, upon the more distal portion of palm or sole, proximal to the bases of the separate digits, and corresponding to the intervals between them.

One Thenar, upon the thenar eminence, *i.e.*, the more proximal portion of the palm or sole, on the side of digit I., radial or tibial.

One Hypothenar, upon the hypothenar eminence, *i.e.*, the more proximal portion of the palm or sole, on the side of digit V., ulnar or fibular.

All eleven of these may be represented in a single palm or sole (H. H. Wilder, 1908, Figs. 1 and 2), but this is the greatest of rarities, only two cases, and these duplicate twins, have been thus far recorded. In the overwhelming majority of individuals the palm and sole patterns present but a small part of the complete set, and exhibit these in every stage of degeneracy down to a slight convergence of ridges in one spot, which indicates the final disappearance of the last triradius. Some of these are much more constant than others, as illustrated by the five apical patterns, the "finger-prints" of the professional dactyloscopists, although even here there is a well-known type, the "simple arch," in which the original pattern is reduced to its lowest terms, and shows merely the position, or "core" of the former pattern without more than the last vestiges of a single triradius. On the other hand, others, like the second interdigital of the hand, or the thenar of the foot, are seldom found, and when they do occur, it is usually in the form of a mere vestige, where the closer approximation, or the change of direction, of a few ridges, are the only indication of the former presence of a pattern that has become lost.

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[1] Contributions from the department of Zoölogy, Smith College, No. 130.

Again, a given pattern, when compared in different individuals in which it occurs, may show every stage of degeneracy, from a concentric whorl, the most complete and ancestral form, through those showing the loss of one or more of the triradii, to a wholly vestigial condition, where a scarcely perceptible disturbance in otherwise parallel ridges is indicative of the last traces of its presence.

In this difference in the liability of occurrence of the different patterns, and in the sort of pattern when it does occur, whether more frequently a primitive Whorl or simply a vestige, we find a perfect correlation with the physiological use of the region involved. In general those patterns which are situated upon the more prominent surfaces, and which are therefore more often in contact with external objects, are far more constant in their occurrence, and appear more frequently in the form of whorls, than are those which, during the normal course of human activities, lie in more retired spots, and are somewhat more shielded. Whorls are, for instance, by no means uncommon on the finger-balls, where a pattern of some sort is seldom entirely lacking, precisely upon those surfaces which are subject to the most wear and tear, and which press the most constantly upon external surfaces; while the four interdigitals are far less constant, and among these the second interdigital, especially protected both by the activity of the thumb, and by that of the coöperating index finger, seldom appears. On the foot the most common place for a whorl is upon the ball of the great toe (the first interdigital pattern) which bears the weight of the body at each forward step, and in the bare foot is constantly in contact with the ground.

Here the pattern is not only huge in area, and composed of coarse ridges, but is very often still in the form of the primitive whorl, composed of concentric circles, and embraced with three triradii. The thenar, on the other hand, lying on the inner side of the foot, is of all foot patterns the most obscure and frequently entirely overlooked. It seldom or never shows more than one or two triradii, and has not yet been reported in the whorl form.

In short, the correlation between the occurrence of the various patterns of the human palm and sole and their varied experiences during the customary activities of everyday life are so great that, could we suppose the entire species to become extinct, and to have left behind absolutely nothing but the records of numberless palms and soles, the customary uses of the lost hands and feet, even to the differentiation between the separate fingers, could readily be surmised and described with much detail.

While it is undeniable that this sounds strongly Lamarckian, there is nothing here intended other than to state the actual facts; whatever one's personal beliefs are with regard to the inheritance of acquired characters, it is certain that there is a strong *correlation* between the occurrence of patterns and the amount of habitual use of the regions where they occur, and between the habitual use and the percentage of occurrence of the more primitive types of patterns.

We feel that we have established the following correlations between the surface relief, the size of the ridges, the occurrence and type of patterns, and the use of the various regions of the surface.

I. Over the raised areas of the palm or sole, which come consequently into more constant contact with external objects, *i.e.*, have the hardest use:

(*a*) The ridges are the largest and coarsest.
(*b*) Patterns occur more frequently.
(*c*) The patterns show a greater percentage of whorls.

II. Over the hollowed areas, which are thus shielded from contact with external objects:

(*a*) The ridges are the finest and least prominent.
(*b*) Patterns are less likely to occur.
(*c*) When they occur they are more likely to be vestigial, *i.e.*; either arches or loops, seldom whorls.

III. In the more primitive patterns the underlying surface often presents something of the shape of a conical mound, and the center of the cone coincides with the exact center or core of the pattern. The hypothenar pattern of the hand, of frequent occurrence in the European-American race, often shows this particularly well, and in the occasional cases in which this pattern is in the form of concentric circles, it forms a definite mound, rising with each circle as one approaches the center, and at the apex is pointed, like a papilla, so that it may be noticeable in profile (Figs. 1 and 2).

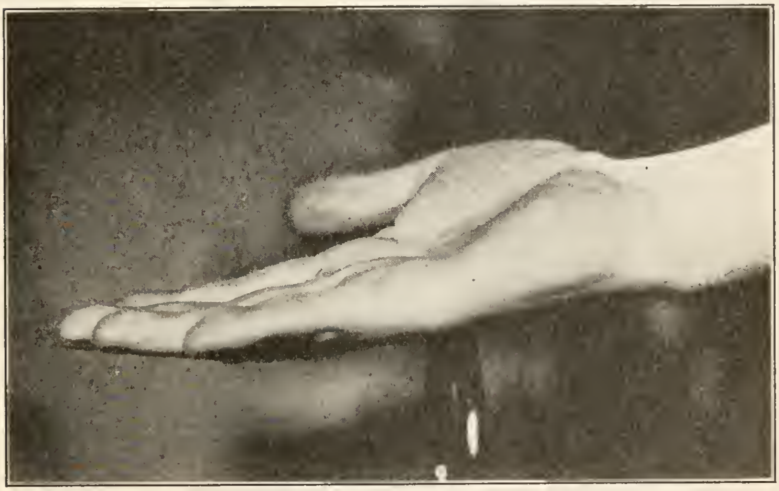

FIG. 1. Photograph of the author's right hand, seen in profile and showing the hypothenar pad covered with a whorl, plainly showing its mound-like relief and the papilla at the core of the pattern.

In spite of the great difference in the percentage of occurrence of the different morphological patterns it is probable that in the human race at the present time no one of the twenty-three patterns of the hand and foot has been allowed to degenerate so completely as never to occur in the primitive form, that of a whorl, although in the more than 1,700 individuals which I have thus far examined, there are at least three places out of the twenty-three in which such a pattern has not yet been found. These, as may be expected, are all in places where there is little contact. Two are in the foot, the thenar, and the hypothenar, and one is in the hand, the second interdigital. Of those in the foot the thenar occurs in the hollow on the inner side of the foot, where only occasionally a piece of clay, a stone, or a wad of grass will ever touch this region, and the hypothenar lies on the outside, and does not meet much contact, although it would be expected, and might well be looked for in the larger Anthropoids.

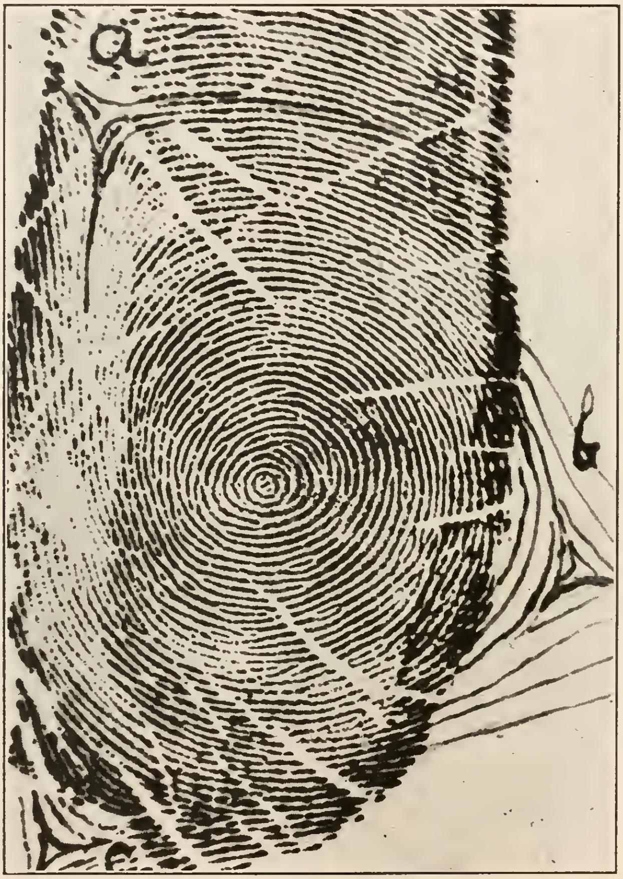

FIG. 2. A very primitive hypothenar of the right hand. Note the three embracing triradii; of these (*a*) is distal, (*b*) is the outer one, and (*c*) the proximal one, on or near the wrist.

Hands.

Thus, to begin with, the *apical patterns*, the ones which are found on the balls of the fingers, that is, the "finger-prints" of the identification experts, are frequently found in the form of whorls. The formulation according to the Henry system, of a given hand as 32/32, means that there is a whorl on every finger. This type is not rare in a large collection, like the one in New York, although to the professional finger-print expert a number of patterns might easily be determined as whorls, which are not typical enough for the morphologist, and would not consist of concentric circles, although they probably would have two triradii. There is some feeling that the presence of whorls upon all the fingers is a racial characteristic of the Jewish race, although definite statistics are not as yet available. Certain fingers are more apt to show whorls than others; for example the thumb and index are very apt to have whorls, while they are seldom found in the ring and little fingers. If one stop to consider the relative amount of independent use, and the variation to which they are subjected, he will see that here, also, as elsewhere, there is a direct correlation between the occurrence of whorls and the amount of use. Still, if one consult a large collection he will have little difficulty in finding even a perfect whorl on a little finger.

Morphologically we have the right to expect *four interdigital patterns*, placed along the distal border of the palm, beneath (proximal to) the four intervals between the five fingers. Occasionally we find the last two, III. and IV., beneath the separations between the middle and ring fingers, and between the ring and little fingers respectively. Thus in Fig. 3, which shows the print of the left hand of a man in New York, one of the finger-print experts at the Headquarters office, 300 Mulberry St., interdigital IV. is particularly well shown, a whorl with a center of concentric circles, and with three triradii, upper, outer, and inner. In formulating this palm either the upper or the outer of these could be used equally well as the starting point of line *D*, with an exponent letter (*t*) to signify an extra triradius. Interdigital III. is also indicated, but not so completely, and with only one triradius, the pattern being simply a loop. Fig. 4, enlarged photographically from the same, gives these same patterns in

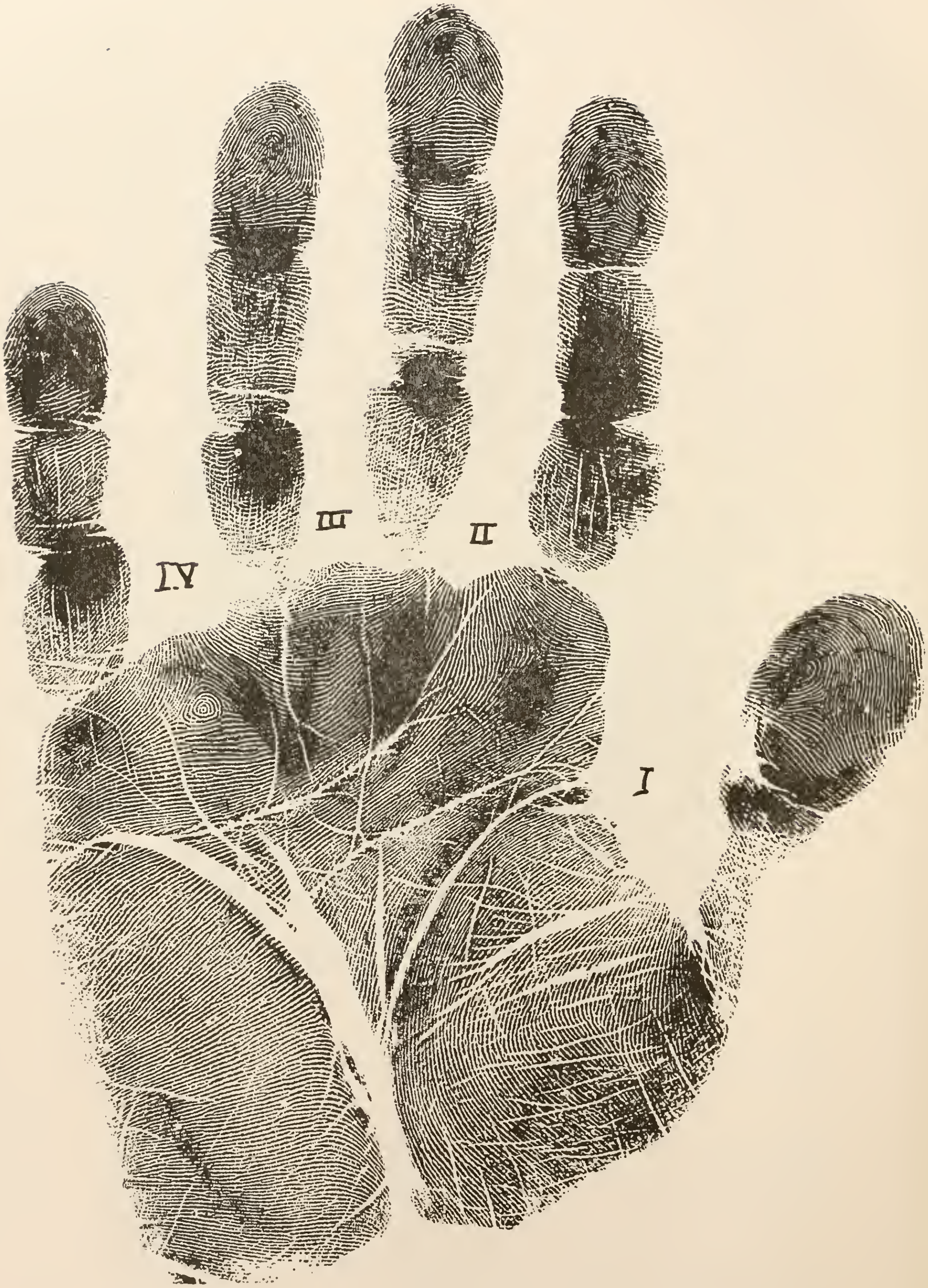

FIG. 3. Hand-print of one of the finger-print experts in the New York office, showing the fourth interdigital in the form of a primitive whorl with three triradii. The third interdigital also is well indicated, but is not an actual whorl, and lacks two of the three necessary triradii.

more detail. In this is seen better the almost perfect condition of the fourth interdigital, with its three triradii. Fig. 5 shows a detail of the right palm of the author's wife (No. 70) with interdigitals III. and IV., the first with two triradii, the latter a rudiment. In the actual hand these two areas are especially prominent, appearing almost like minute papillæ, with the patterns on their apices. In the left hand of the same individual (Fig. 6) the same two interdigitals are shown, but only one of them is a whorl, the IIId, while the IVth is simply a loop, a very common pattern in the European-American race, and almost usual enough to serve as a racial characteristic in Japanese and Chinese.

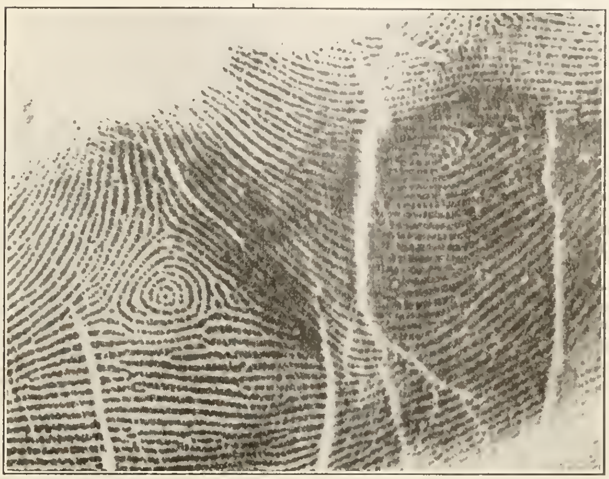

FIG. 4. Detail taken from Fig. 3, enlarged. Here the third interdigital pattern is very typical, and compares well with the primitive hypothenar shown in Fig. 2.

Occasionally one meets with either the third or the fourth interdigital as perfect as in Figs. 3 and 4, but it is always a surprise. My first experience of this kind was in one of two twin girls, relatives of the Director of the Eugenics Record Office at Cold Spring Harbor, L. I., but not found in her sister. This was in the right hand, and showed a whorl with three triradii, in the position of the IIId interdigital (Fig. 7). In enlargement (Fig. 8) it shows the three triradii. Fig. 9, a sketch taken with an Abbe camera, shows the details of the separate ridges. The corre-

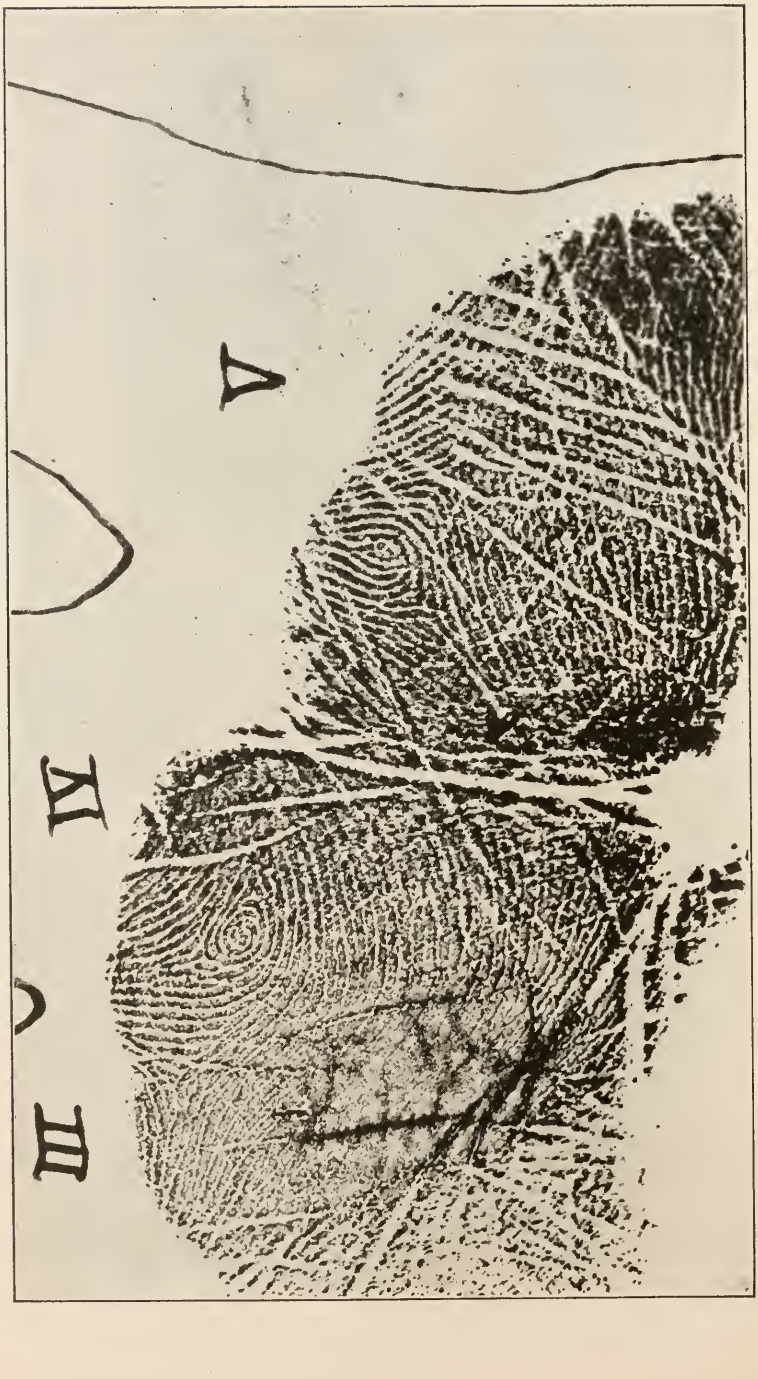

FIG. 5. Print of the distal portion of the palm of No. 70, showing interdigital patterns III. and IV., distinct but small. Compare with Fig. 6, which shows the other palm of the same individual. These show in the actual hand the same distinctness described under this other figure.

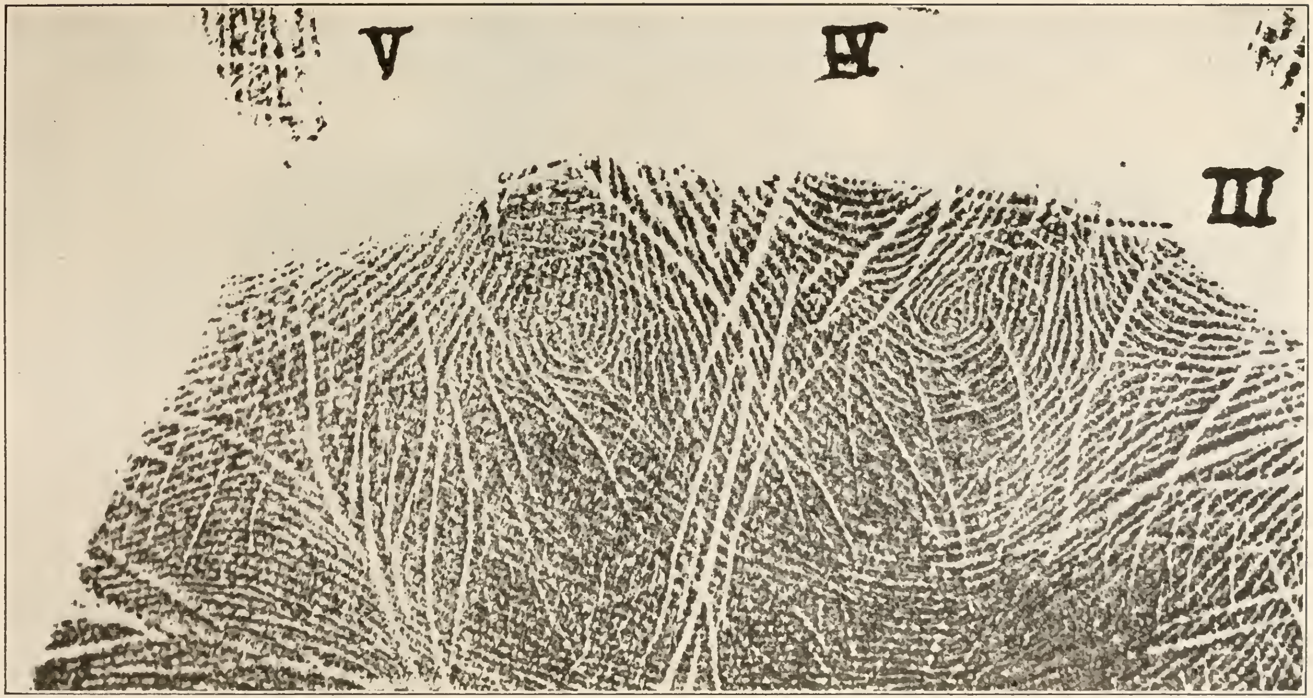

FIG. 6. Print of the distal portion of the left palm of No. 70, showing interdigital patterns III. and IV., distinct but small. In the actual palm these patterns form the apices of little low papillæ, which, when looked at across the surface of the palm, especially in the right light, appear as definite organs.

sponding IIId interdigital of the left hand of the same person (Fig. 10) shows an indication of the same pattern, but not nearly

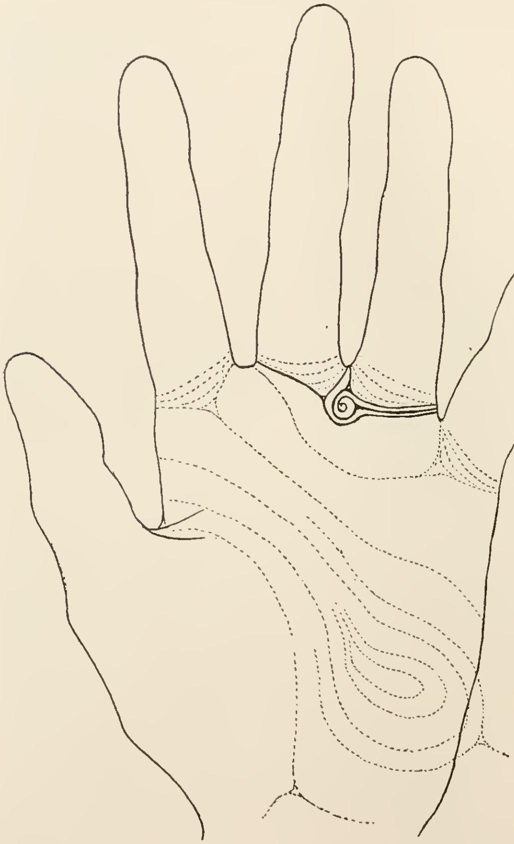

FIG. 7. Tracing of the right hand of No. 1056, with a conspicuous third interdigital pattern. The left hand of this same individual shows a good whorl on the same place (third interdigital) but smaller, and not so clear. Compare, for details Figs. 8 and 9.

as complete. Other cases of the occurrence of these interdigital patterns, the IIId and the IVth, are shown in Figs. 11 and 12, and include nearly every case I have met with. Occasionally, as in Fig. 11 the pattern is minute, but may still retain two out of the three triradii; in other cases, the pattern is still more shrunken, and can be identified only by its position on the palm, as in Fig. 12. Even here, however, in its reduced state of one

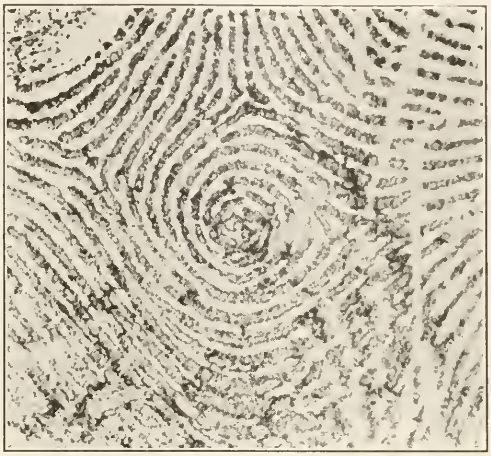

FIG. 8. Enlarged photograph of the third right interdigital pattern of No. 1056. The entire hand, with position of this pattern, is seen in Fig. 7.

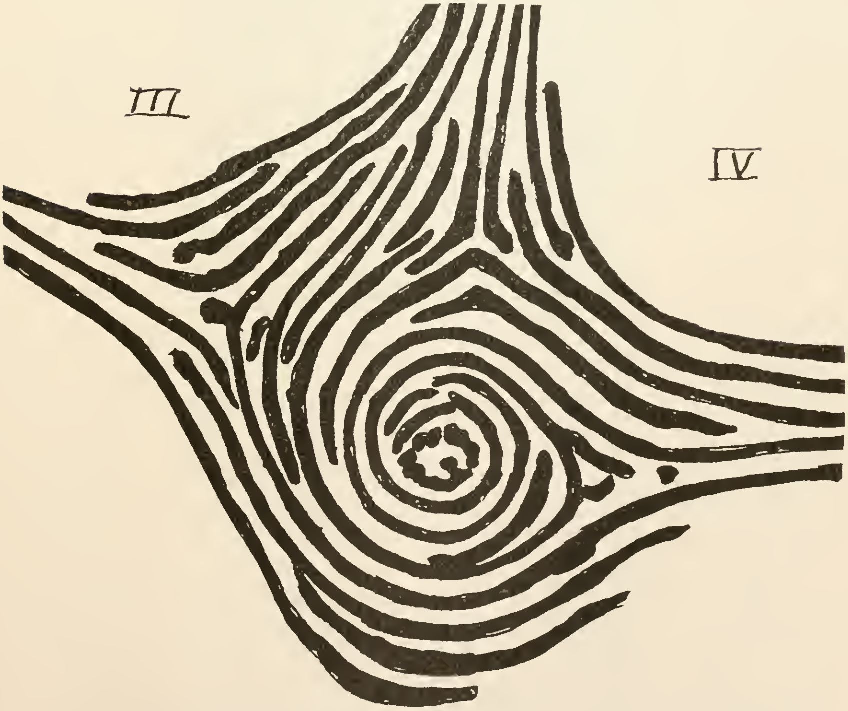

FIG. 9. Detail of the third Interdigital pattern of the right hand of No. 1056. Compare with the photograph of the same, shown in Fig. 8 and the tracing of the entire hand of Fig. 7. Drawn from an original print by a drawing camera.

ridge forming a circle, with a dot in the center, the reduction to its lowest terms, there is at least one of the three triradii, and a possibility of treating a prominent fork as another one.

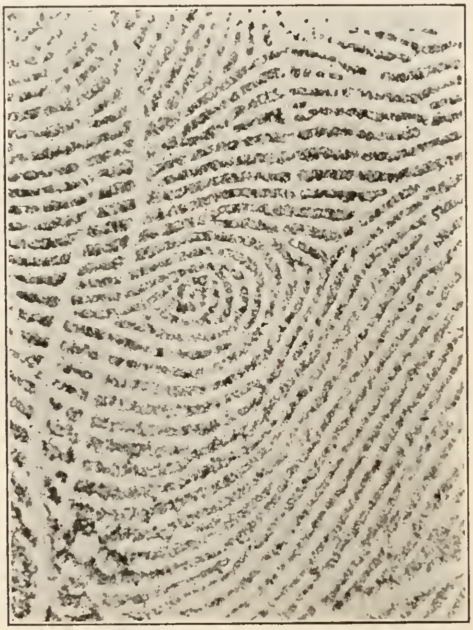

Fig. 10. Photographic enlargement of the left interdigital pattern of No. 1056. Compare with Figs. 6 and 7.

Interdigital II., between the index finger and the middle one, is always rare and never more than a loop at best. In hands looked over for this very pattern, my collection gives the following:

European-Americans (females)	200 hands	5 cases
Japanese (males)	166 hands	7 cases
Japanese (females)	224 hands	2 cases
Chinese (equal number of males and females)	200 hands	1 case

Fig. 13, taken from my Japanese collection, gives as good an example of this rare pattern as I have. This hand is interesting as showing also the IIId and a rudiment of a IVth, indicated by the convergence of the ridges in the proper place. This rudimentary condition seen here is also quite likely to occur on the area designated for this pattern. Fig. 14 also from a Japanese male, shows

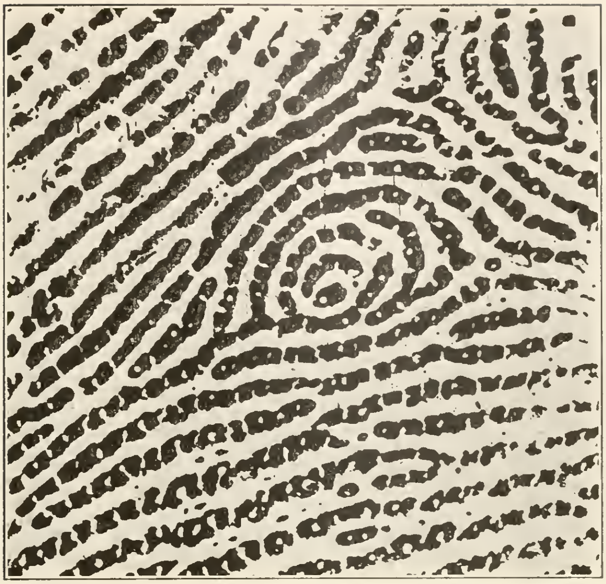

FIG. 11. Photographic enlargement of the fourth interdigital pattern of No. 1212. This pattern is minute, but accurately placed directly below the interval between the ring and little fingers. Of its three triradii, the upper one forms the starting point of line *D*. Line *C* has no triradius in this hand. The photograph was given by Mr. Bert Wentworth, and was taken by the owner of the hand.

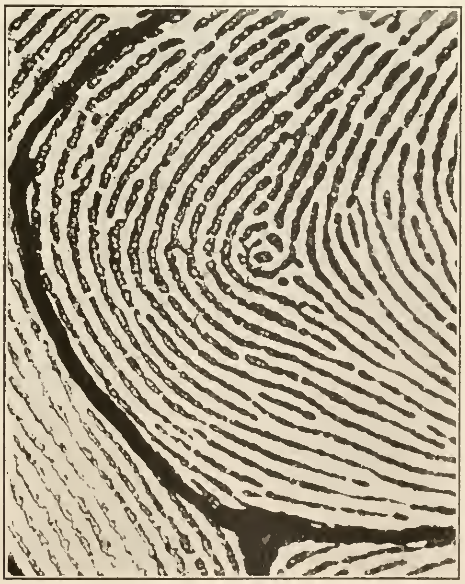

FIG. 12. Photographic enlargement of the right third interdigital pattern of a female, not in my collection. This photograph was given me by Mr. Bert Wentworth.

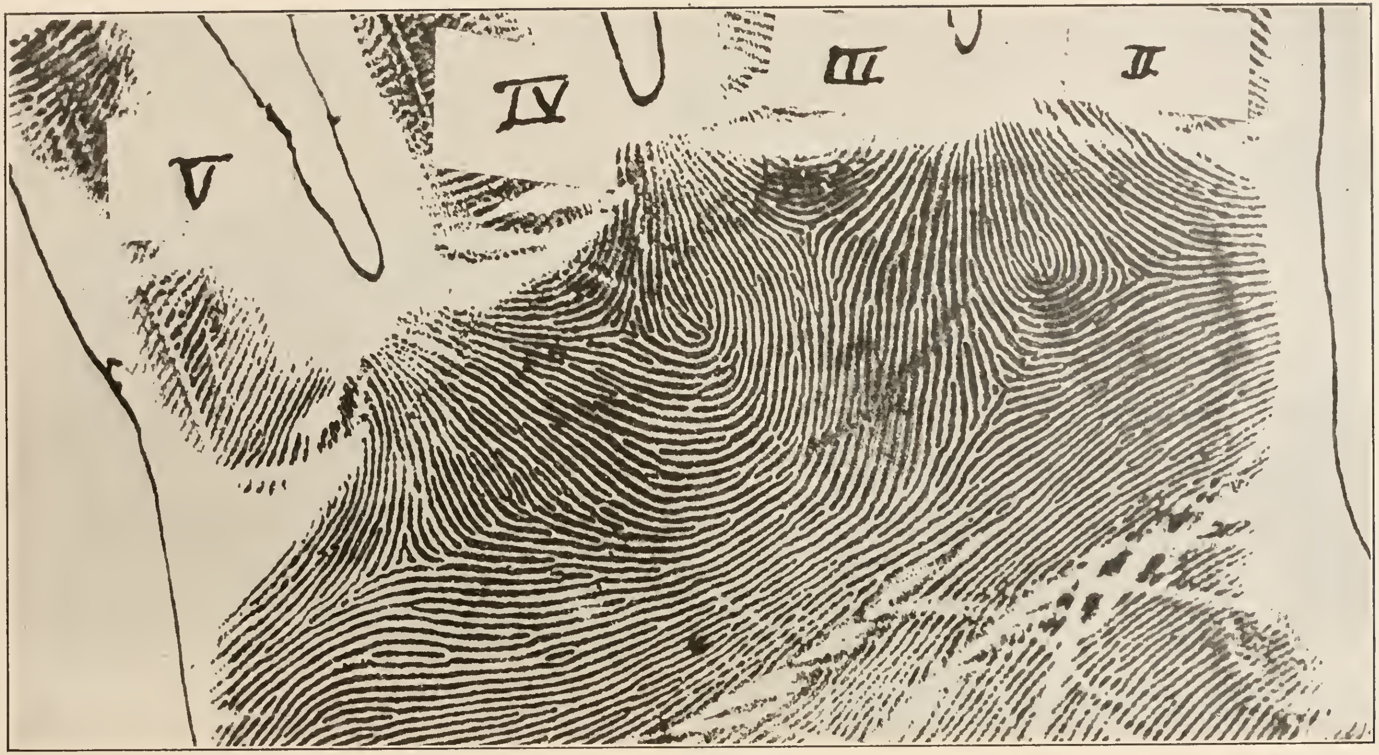

FIG. 13. Print of detail of left hand of No. 1517, Japanese male, showing interdigital II., unusually well-developed, yet not in the form of a whorl; also interdigital III., in the form of a loop, and interdigital IV. as a vestige, with convergence of ridges in the place of the lower triradius.

the convergence of ridges quite definitely and may be considered as a case having this IId interdigital pattern in the condition of a rudiment.

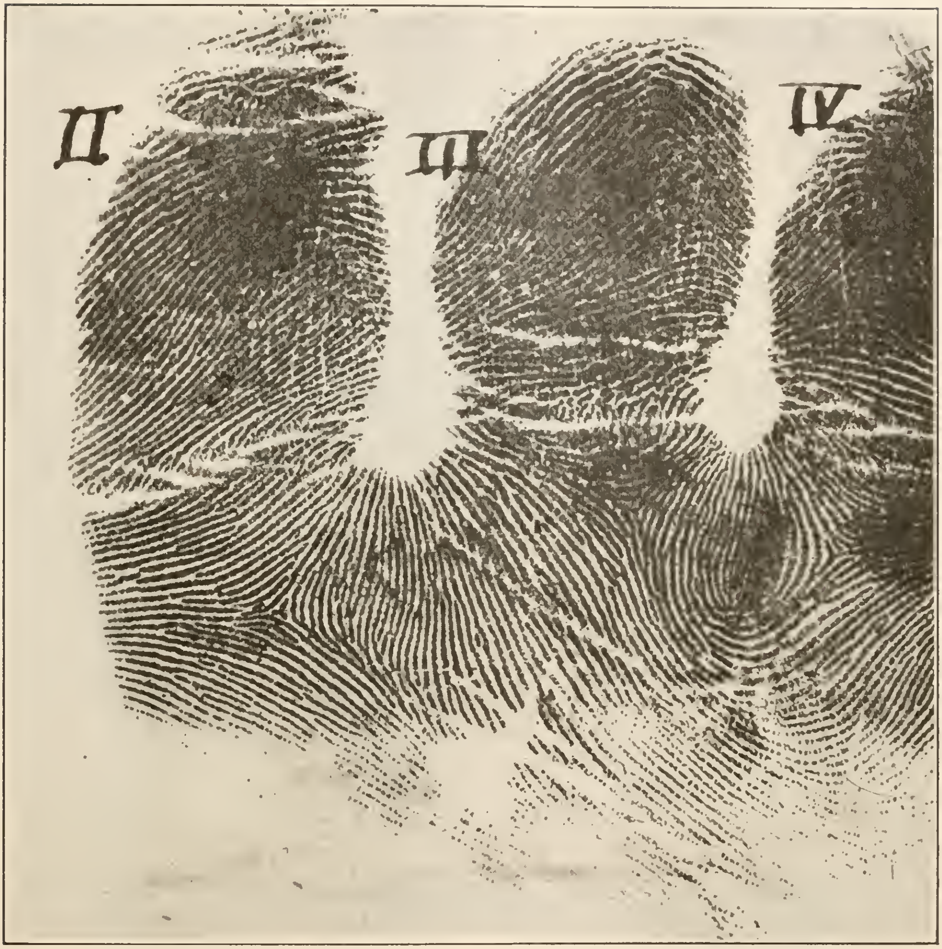

FIG. 14. Detail of print of No. 1585, (Japanese male) showing vestige of interdigital II., between index and medius; also interdigital III. in the form of the more usual loop.

Hasebe finds the percentage of occurrence for this pattern as 2 per cent. among the Japanese, and 4 per cent. among the Aino, corresponding fairly well with my figures.

Interdigital I. has long been, in my experience, in the same class as interdigital II., that is, that no whorl pattern has yet been known, but within a few years a quite perfect whorl, although small, appeared in my collection of prints of college students. The normal close association of this with the definite thenar will be remembered, how in the majority of cases with any disturbance at all in the thenar region, there are apt to be found two loops back to back, and facing in opposite directions; the true thenar and the first interdigital. In the case in which the first inter-

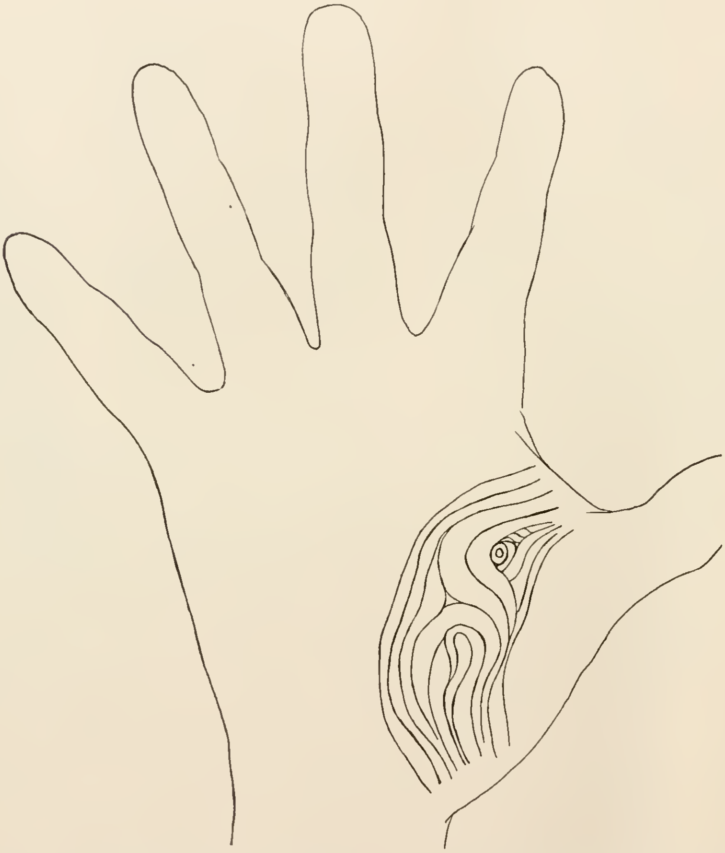

Fig. 15. Tracing from the print of No. 788, a Smith College student of European-American race. This is the only case thus far known of a complete whorl in the position of the first interdigital. This has apparently the full quota of three triradii, but the triradius below, between this pattern, and the thenar, which is here, as usual, represented by a loop, evidently belongs to the thenar pattern.

digital appears as a whorl, as shown in Figs. 15 and 16, the core or center of the pattern is marked by a complete circle, beyond which there are surrounding ridges which assume the usual loop shape, and bear the usual relationship to a thenar. This is certainly not a large or conspicuous pattern, but still answers to the requirements of a definite whorl. It is to be remembered in this case that it occurs in one of the so-called "better classes" of our own race, and that there is otherwise nothing especially primitive in this individual.

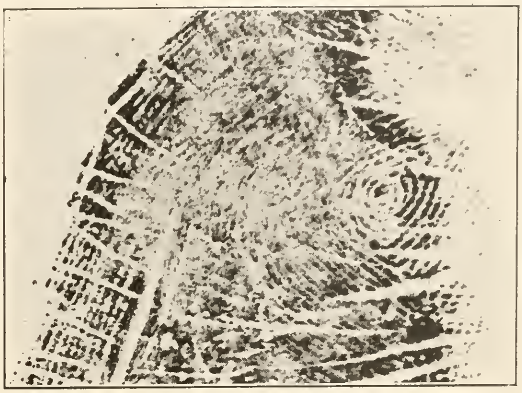

FIG. 16. Detail of the first interdigital pattern, from the left hand of No. 788, a Smith College student. This pattern here forms a complete whorl, though small. It is the only case yet reported in this position.

The case is a bit different in the only case of a thenar whorl which I have seen, as it occurs in a native Liberian soldier, one "Jimmy" Kamo, of the Bande tribe, collected for me by Prof. Frederick Starr (Fig. 17).

This print is in my possession and although it is very dense, as too much ink was used, it is unmistakable. By treating the print with turpentine, as may always be done in such cases, the separate ridges can be well brought out. A second case like this has been published and figured by Hasebe (his Tafel IV., Fig. 10). This author states that he has met a thenar in form of a whorl twice, both times in Japanese.

Hasebe finds the occurrence of any form of thenar pattern as 5 per cent. of all palms in both Japanese and Ainos, which may be compared with what I have found in European-Americans, 7 per cent. in one investigation, and 11 per cent. in another. The percentage is much higher in negroes, and very much higher (50 per cent.) in Maya Indians.

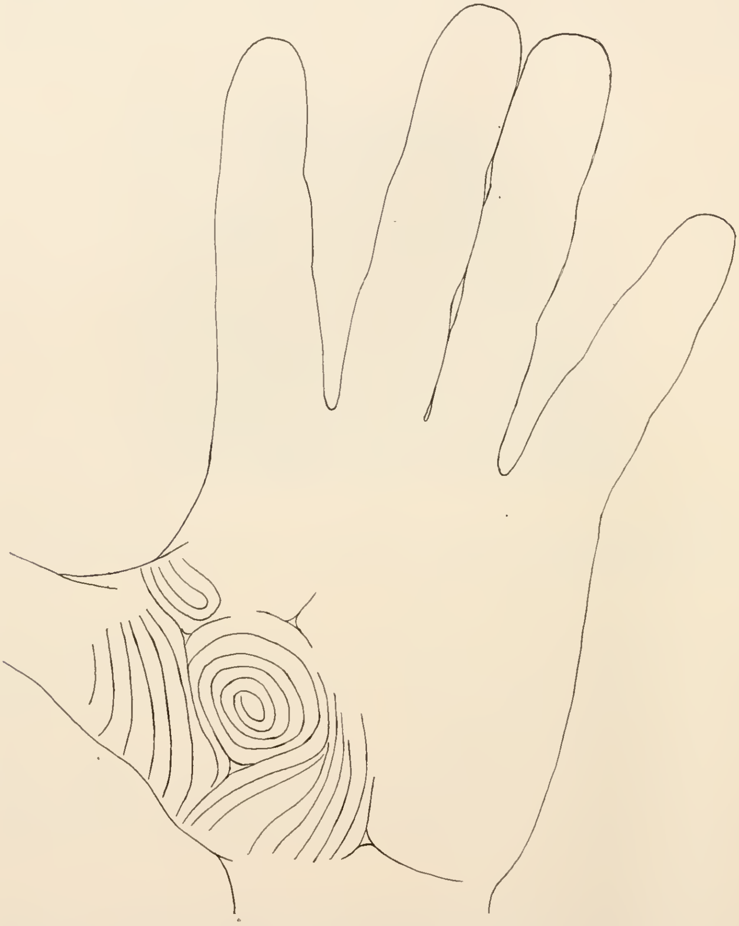

Fig. 17. Tracing of the right hand of No. 571, showing a complete whorl on the thenar eminence. The subject is a Liberian soldier, Jimmie Kamo, of the Bande tribe. The print was taken by Prof. Frederick Starr.

For a time this case of the Liberian soldier, which I published in the *American Anthropologist*, 1913, p. 202, Fig. 35 was the only one known, but Hasebe has recently (1918) found a second one. Thus, with the unique case of my No. 788, the two patterns on the thenar side of the thumb, which represent the closely associated first interdigital and the true thenar, are both occasionally represented as whorls.

Feet.

The study of toe patterns, doubtless owing to the universal deformation of the toe-balls through shoes and stockings, has scarcely been studied in this country or in Europe, but Hasebe, whose countrymen universally wear clogs (geta) which protect the plantar surface from the roughnesses of the soil, yet allow perfectly natural freedom in walking, and encourage the habit of dispensing entirely with all such artificial covering when in the house, has taken advantage of the material thus provided, and gives a careful report on the figures on the balls of the toes, the apical patterns of the foot. He studied the toes of 100 individuals (1,000 toes) and finds the distribution of whorls on the toe-balls as follows:

Right:		Left:	
1st (great)	4	1st (great)	10
2d	18	2d	13
3d	53	3d	50
4th	11	4th	12
5th	0	5th	1

Hasebe further expresses these in the form of percentages, putting both feet together, as follows:

Percentage of Whorls (both feet):	
1st (great)	7.0%
2d	15.5%
3d	51.5%
4th	11.5%
5th	.5%

From these figures there will be seen a general similarity to the condition of the fingers in respect to the occurrence of whorls, except for the small percentage in the great toe when compared with the thumb; yet we would expect this very difference when we consider the amount of difference in the habitual action of the two digits. The thumb, with its extreme flexibility, and the short and rigid great toe, show, in this difference in the occurrence of whorls, at least a correlation between use and morphology, of the same sort as elsewhere in the friction-skin configuration. The large occurrence of whorls on the third toe is unexpected, but may be accounted for by the prominence of that digit in ordinary walking, and its extreme projection in the average foot.

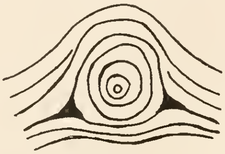

FIG. 18. Apical pattern of the right great toe of a European-American.

Typical whorls do occasionally occur in the apical pattern of the great toe (Fig. 18), but a loop, usually fibular, is much commoner.

Unlike the hand, the foot, owing to the greater equality in its digits, and especially to the evenness of the intervals between them, disposes of its four interdigital patterns in a straight row across the sole, occupying the mounded region commonly spoken of as the "ball." Remembering that the first interdigital pattern is here in line with the others, and is neither dropped out of place, nor approximated to the thenar, as in the hand, there is occasionally a sole in which all four interdigital patterns appear as either loops or whorls, but in a number of cases, perhaps the majority, the interdigital areas except the first are not marked by patterns,

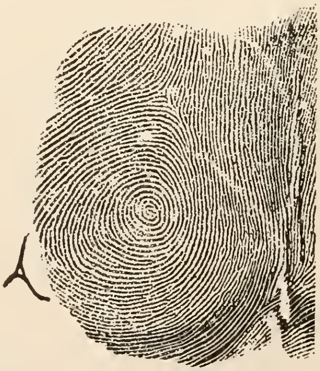

FIG. 19. First interdigital (hallucal) of right foot of a Japanese girl, a student at the Doshisha school in Kyoto, showing the concentric whorl of a primitive pattern. The outer triradius was not printed but its position is indicated by the convergence of the ridges. It would doubtless have been printed if the foot had been rolled a little.

but in these cases the areas where they belong are sufficiently well indicated by the triradii, especially the upper or distal ones. The first interdigital area is often marked by a whorl, frequently a typical one (Fig. 19) but the three others are either in the form of a loop, which may open either up or down, or, perhaps most frequently of all, are crossed by approximately parallel lines with no suggestion of a pattern. The second interdigital pattern often gives the suggestion of having been squeezed laterally, and is often in the form of a narrow loop, opening downward. Aside from the first, the third interdigital is the most likely to be in the form of a whorl (Fig. 20), and in Fig. 21, there is a large whorl, concerning whose identification there is some doubt, not cleared up by the tracing of the entire sole (Fig. 22), although it is probably the fourth.

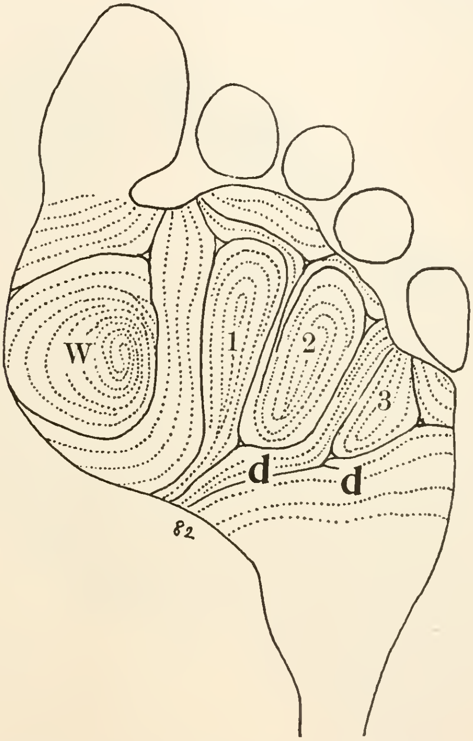

FIG. 20. Tracing from a sole print (No. 82) showing the areas of the four interdigital patterns. The first and the third are seen as whorls.

Thus, if this last be taken as the fourth interdigital, and the whorl of Fig. 20 be considered the third, which is very evident, then, with the frequent occurrence of a whorl pattern on the first interdigital, or hallucal, this leaves the second alone which has not yet been seen in the primitive form, and the squeezed up condition of this renders it very unlikely that it has in modern men any longer the chance to express itself. It is quite likely that sometime the impress of a naked foot of some paleolithic man may be found on the clay floor of a European cave, and it will be then with the most breathless interest that we will look to see if the second interdigital area was then as pressed laterally as now, or whether it ever expressed itself in the form of a whorl.

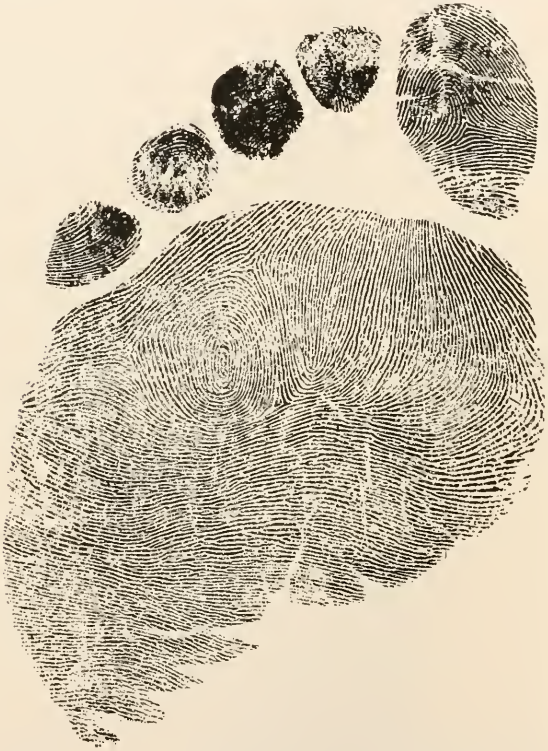

FIG. 21. Sole print of No. 712, in which the third interdigital pattern, a whorl, is present, and large. Compare with Fig. 22, a tracing of the same sole.

There is still some little doubt concerning the identity of the proximal part of the foot, that proximal to the line of interdigital patterns which runs across the ball, but, after the analogy of the hand the *hypothenar pattern* is to be looked for somewhere along the outer, or fibular, edge of the sole, a little back of (proximal to) the interdigital pattern of the little toe and the one next to it. There is frequently found a loop in precisely this place, which runs across the entire sole, the core of which is so far out that it is usually beyond the tread area, and requires a slight outward roll of the foot to get wholly into a print. Generally, too, the loop is a narrow one, with a straight axis, but occasionally one may be met with that has a definite bend in the axis, making the loop into a hook, as in the Negro boy in Fig. 23. In one case I have found this bending of the loop so extreme that the core assumes almost the appearance of a whorl (Fig. 24). This occurred on the foot of a small son of a university professor.

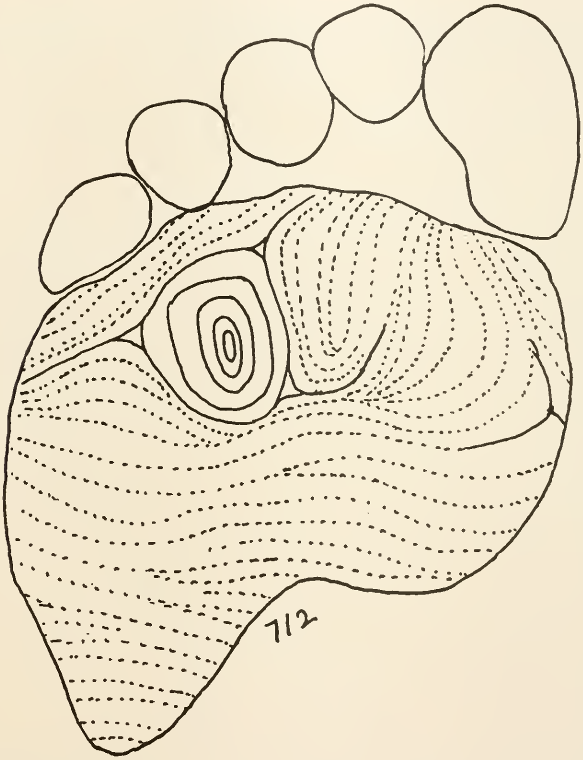

FIG. 22. Tracing of the sole print of No. 712, shown in Fig. 21. The whorl is probably that of the fourth interdigital pattern.

The thenar pattern is to be expected on the inner side of the foot, proximal to the first interdigital pattern. This would place it on the hollow of the foot, precisely where the surface is seldom touched by foreign objects. We might expect, *a priori*, not to find the pattern developed, but to find, at most, a triradius or two, and a patch of ridges in a different direction. That is precisely what we do find, if we take the precaution to print a separate piece of paper, by first inking the inner curve of the foot directly, and covering it with the piece of paper. In the majority of cases the result is negative, but occasionally there is some remnant of a pattern, and this is exactly what one would expect, quite frequently a loop, either with or without a triradius, and

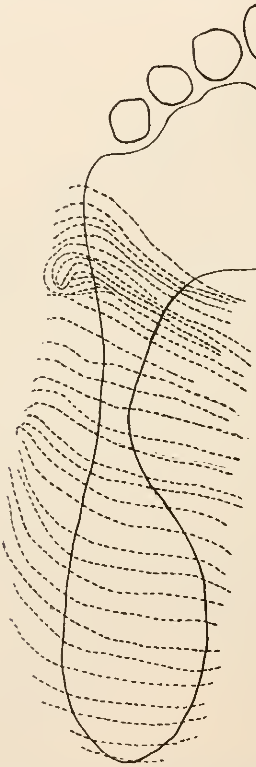

FIG. 23. Tracing of No. 178, a Negro boy, with an unusually primitive hypothenar pattern. The loop, which is unusual, has become bent backwards, or recurved, leading towards the still more primitive one shown in Fig. 24, which has almost become a whorl.

always drawn with very fine and soft ridges. The difference in this feature between members of the European race, on the one hand and Japanese and Chinese on the other is sufficient to serve as a racial criterion, for the almost entire absence of any trace of such a pattern in the latter, and the frequency of some trace in the former is very striking, as is shown in tabular form in my recent paper on the Chinese and Japanese (*Journ. Phys. Anthrop.*, Vol. V., No. 2, p. 203, 204).

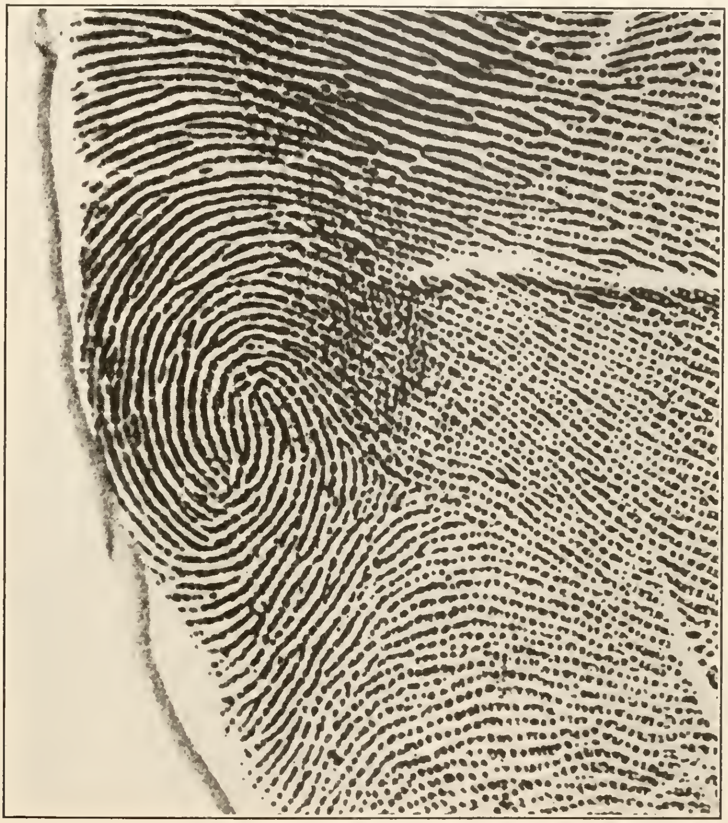

FIG. 24. Left hypothenar pattern of the foot of No. 1107. In this boy who is of the European-American race, the pattern has gone in the same way as in the Negro boy (No. 23), and has produced the nearest pattern to a whorl that has yet been found in any race.

The calcar pattern completes the study of the feet. This is of rare occurrence in any race, but has been observed in several cases. It occurs about once in every hundred individuals among our people, and is usually in the form of a large loop, covering almost the entire heel, opening to the tibial side (Fig. 26). A triradius is usual, though not necessary, lying near the curve of the loop. I have met with this pattern a few times, notably in each of a pair of duplicate twins, as was to be expected. Naturally it would be most unexpected to find this pattern in the form of a complete loop, but one such case has been found, in the foot of a university professor (Fig. 27).

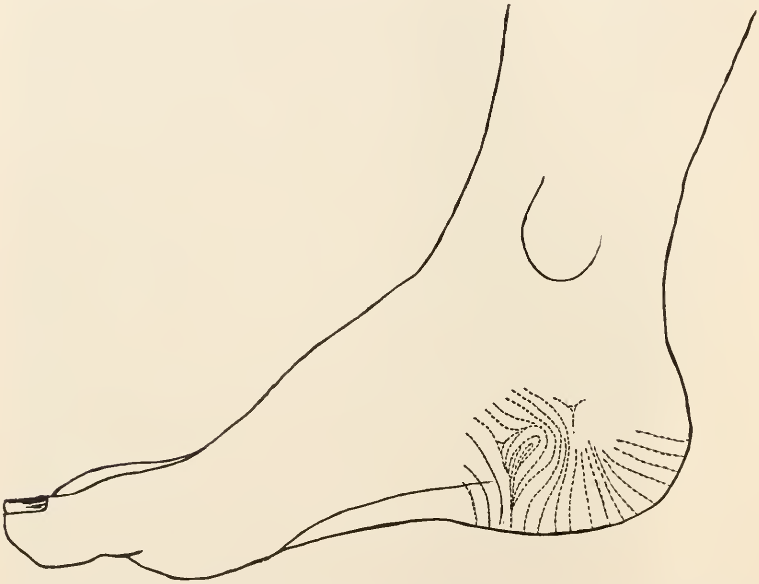

FIG. 25. Foot of No. 87, showing the thenar pattern in the form of a closed loop. One triradius is plainly visible, the upper one belongs on the curve of the loop, and its position is sketched in (dotted line). The third, which is necessary to transform the figure into a whorl, is indicated by the convergence of the lines of the loop. That this figure is not a misplaced calcar pattern is shown by the fact that in this same foot there is a normal calcar pattern, of which the end is shown by the convergence from the other side of the ridges that come around the heel. The friction-ridge configuration is taken directly from a print, but the foot outlines are conventional, and serve to show the position of the pattern.

The morphology of the calcar pattern is still uncertain. It occurs on the calcar projection of the heel, which is distinctly human, and is unrepresented in the foot of any of our other Primates, even the anthropoid apes. One theory identifies it with a pattern in the form of a loop, which is quite constant in the lower anthropoids, but the feet of these forms, although carefully studied, has not been treated with the basis of the walking-pads as a background and is not wholly satisfactory. Another theory links it with the hypothenar, and treats it as a component part of an enormously extended pattern, like the one reported in a few cases, mainly of negroes, which covers almost the entire sole, back of the ball. It is also conceivable to connect it with the vestiges of the thenar. The finding of a complete calcar pattern, as in Fig. 27 goes a long way towards the determination of the calcar as an independent pattern, distinct from all others, and developed secondarily upon the extensive human addition.

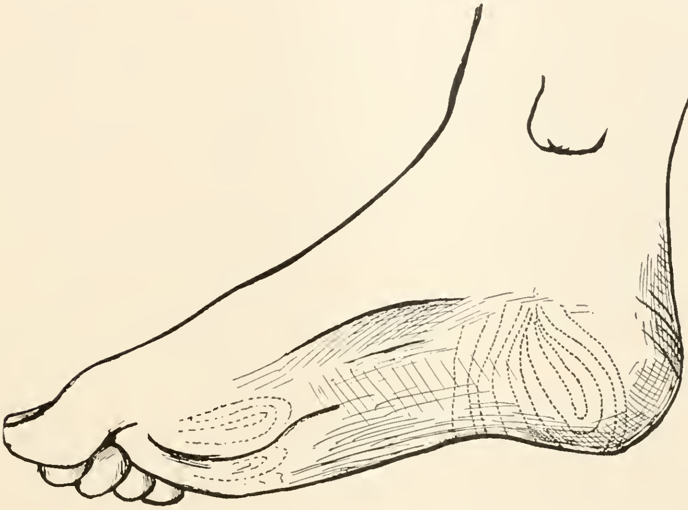

FIG. 26. A typical calcar pattern in its usual form, that of a loop, with the head of the curve fibular, and opening (*i.e.*, converging) towards the tibial side. From life, No. 1128, pattern drawn in with help of prints.

The study of comparative morphology is replete with instances of survivals; the persistence of organs of former usefulness later allowed to lapse from a growing lack of importance until, no longer of value, they become vestigial. The original eleven walking pads of the primitive mammalian paw, still useful and constant in pentadactylous forms like squirrels and mice, and retained in the form of whorls in the pictures sketched by the Primates in friction-ridge patterns, and faithfully representing a former relief of pads with their surrounding skin folds and embracing triradii, and these, in spite of the high intellectual development of man, with its accompanying change in life and habits, will still occasionally crop out, not especially in those races commonly considered low and bestial, but are found quite as frequently in peoples of the highest culture, and appear in primitive form now in the foot of a university professor, or in the hand of the daughter of a New Hampshire bank president, of a

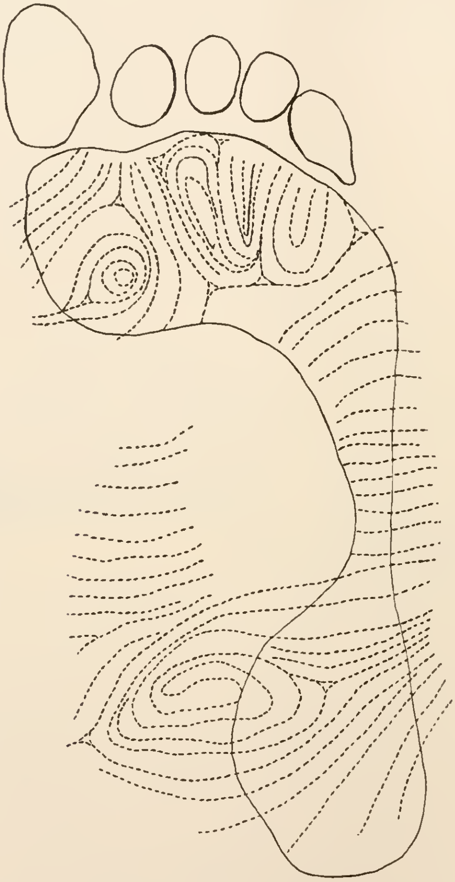

FIG. 27. Outlines taken directly from sole prints of No. 887. This sketch is taken from several prints, lapped over so that they will meet, and spread out flat. This device is rendered necessary in order to express on a flat surface the details of a curved one.

New York finger-print expert, or of the author of this paper. It is true that one of the most primitive illustrations was found in a native Liberian soldier, but equally primitive cases, both in hands and feet, occur among the students of Smith College.

In my rather limited collection of peoples generally considered primitive, and including Ainus, Bontoc Igorots, Negritoes, and African Pygmies, I have found no cases that compare for primitiveness with those I have figured, which, with a few exceptions, were all of the European-American race. In some cases, if not in all, however, the lack of primitive characters has been undoubtedly due to the small number of individuals in these exotic cases, although, in the cases of the Japanese and Chinese, I was enabled to consult a sufficient number of cases to bring out unusual cases if there were any. Still, we know as yet too few individuals of any race, even of our own, to draw any sweeping conclusions from them, and it is best to treat the cases here presented as individual rather than racial.

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In my paper of 1916, published in this magazine, I endeavored, as was fitting a relative new subject, to make the bibliography as near complete as possible. Since then, with the added interest in this field, the task of keeping even the titles complete is quite beyond me, and nowhere near as possible as it was nine years ago. Here I may content myself with adding a few of the most recent titles, from the bibliographies in which one may obtain the later literature.

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MORPHOLOGY AND LIFE HISTORY OF *POLYTOMELLA CITRI* SP. NOV.

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The organism dealt with in this paper was first found in Berkeley, California, during January, 1924, in a culture medium for *Euglena gracilis* made after Zumstein which contained the following parts: peptone .5, citric acid .2, grape sugar .5, $MgSO_4 \cdot 7H_2O$ .02, KH_2PO_4 .05, water 100. In September, 1924, a culture was shipped to me in New Jersey, from Berkeley and the cultures made from this material are still in good condition.

A little observation showed that this flagellate reproduces very rapidly during the active stage and also that an abundance of resting forms are periodically present. These facts seemed to indicate possibilities for studying the life history. Since no previous account of this organism has appeared, the name *Polytomella citri* is proposed for it.

I wish to thank Professor C. A. Kofoid for the many courtesies extended to me while working in his laboratory, Professor E. Newton Harvey for many valuable suggestions, and Professor E. G. Conklin for his constant guidance and advice.

Methods.

Polytomella citri thrives on the medium mentioned above, but it does not make a lasting culture, two weeks usually being the limit. The best results were obtained from uncooked timothy hay in distilled water, to which a little sugar was occasionally added. An old culture can easily be renewed by dilution with distilled water. Successful cultures can be made on media with P_h value ranging from 3.5 to 9. The P_h of old hay cultures is always around 5.5 which seems to be the ideal value for this protozoan. On several occasions fresh cultures were placed in a refrigerator. After remaining there for two months the jars were fairly teeming with *Polytomella*.

During the early part of the work the cultures contained in addition to *Polytomella*, *Glauçoma scintillans*, *Chilomonas paramecium*, *Euglena gracilis*, *Bodo* sp. and some mold, but after several months everything disappeared except *Polytomella* and the mold. Zumstein's medium showed only *E. gracilis* mold, and *Polytomella citri*, the acidity of this solution being too great for the others.

The active forms were fixed in hot Schaudinn's fluid and stained in hot iron-alum-hæmatoxylin. Methyl green and Delafield's hæmatoxylin were also used, but with rather poor results. For counterstaining Bordeaux red, eosin, methylene blue, and orange G were employed, the first two yielding by far the best results. Eosin was used in the 95 per cent. alcohol, while when Bordeaux red was the counterstain the slides were placed in a 0.1 per cent. solution of the dye for twenty-four hours, immediately after applying the hæmatoxylin. Bonney's triple stain was tried, but was found to have little value for this work.

Most of the preparations were made by pipetting a drop of the culture fluid onto a slide, which had been previously smeared with a little albumen fixative, permitting this to evaporate until it did not run and then dropping slide in Schaudinn's for two minutes. Although this technique was quite successful even better results were gotten by centrifuging the material and fixing before pipetting onto the slides.

It was found that the encysted forms did not fix well in Schaudinn's nor was it possible to get them to take any kind of stain, even aceto-carmine failed to leave any trace of color. The successful technique finally developed was fixation in Bouin's or McClung's modification of Bouin's for from twelve to twenty-four hours, followed by dehydration and imbedding in paraffin. Sections were cut from two to four micra thick, and stained as above. Schaudinn's fluid was tried in place of Bouin's, but usually resulted in shrinkage of the protoplasm away from the cellulose wall.

MORPHOLOGY.

Polytomella citri is a colorless flagellate of pyriform shape, the anterior end being rounded, the posterior rather bluntly pointed. The size varies from 14 by 10 micra to 10 by 7, while the average dimensions are 12 by 8 micra. Although the body of living individuals is usually pear-shaped, the form can readily be changed. Thus we find some whose width is greater than their length, others with their posterior ends split into two or more pointed processes (Figs. 2, 3 and 4). In the latter case a groove extends forward for some distance from the notch between these processes. An optical cross-section of the typical form would be circular.

The cell is enclosed in a very thin pellicle. This covering is not visible in life and it divides with the body at fission. It is also to be noticed that it is not sufficiently rigid to prevent considerable changes of shape (Figs. 2 to 4). However, its presence can be demonstrated by dissolving the contents of the cell with dilute NaOH. When this is done the pellicle can be seen, providing the light is cut very low. In some cases plasmolysis causes the protoplasm to shrink away from the wall, thereby serving as a demonstration of its existence, but it requires very careful manipulation. The composition of this covering could not be determined, but since the heavy wall which develops around the cyst was found to be cellulose it is presumed that this has the same chemical constitution.

At the center of the anterior end are two very noticeable basal granules from which arise four equal flagella. These are slightly shorter than the body and do not taper at all, being as large at the free end as at the point of insertion. The two pairs of flagella are well separated in the living organism by a small cytoplasmic protuberance which extends forward between the basal granules (Fig. 1). This prominence is never preserved after fixation.

The two contractile vacuoles are located at the anterior end. They pulsate alternately.

The spherical nucleus is about 3 micra in diameter and is situated at any point along the long axis of the cell from the posterior fourth to the anterior fourth. All of the chromatin is concentrated in a central sphere, the karyosome. This body is anchored to the nuclear membrane by an indefinite number of achromatic strands. Six is the greatest number of these supports that have been seen, four the least. Aside from the strands the space between the karyosome and membrane is optically empty.

Surrounding the nucleus is an area of very granular, slightly chromatic cytoplasm. This is of very variable extensiveness, sometimes filling half of the cell, while in others it is hardly noticeable.

Examination of living specimens reveals longitudinal striations which number about eighteen. It was not possible to make sure of the exact number and consequently their constancy could not be determined. Superficial examination would lead one to think that these were on the outside of the pellicle, but careful focusing shows that they are slightly below the surface and that they do not quite reach either the anterior or posterior ends. They, consequently, must be either on the inside of the pellicle or in the outermost layer of the ectoplasm. After devoting considerable study to this question I rather lean towards the latter, and think that the striated effect is produced by some stringy arrangement of the protoplasm. Living mounts or iodine-eosin preparations show this structure very well, but fixed and stained material gives no indication of it.

In the living cell all of the internal structure is effectually screened by relatively large starch bodies which are located peripherally. They may be so large and numerous that they are packed closely together from one end to the other or they may be entirely absent. By the use of a medium which does not contain any carbohydrate (peptone 0.5 part, citric acid 0.2 part, magnesium sulphate hydrate .02, KH_2PO_4 .05 part, and water 100 parts) the organisms may be freed from starch for several days, when it reappears again. No explanation of this fact is known. The addition of cane-sugar to a timothy hay culture will increase the amount of reserve starch in the cells. If they are only partially filled with these bodies it is generally the posterior end which is free from them.

The following reactions are the justification for identifying this material as starch; it gives a deep blue color when treated with iodine; it is digested by ptyalin; when treated with iodine and heated it loses its color, the blue returning when cooled; when treated with iodine followed by NaOH no color is found.

The presence of a centriole could not be demonstrated in the interkinetic cell of the active form. However it was seen in several of the division stages figured in this paper (Figs. 13–15). Sections of cysts showed a centriole very clearly.

In the active forms an indication of rhizoplasts connecting the basal granules with the nucleus was seen in a few cases (Figs. 20 and 24). This, however must remain purely tentative until more evidence can be produced. There is no doubt as to the presence of this structure in the cyst just previous to excystment (Figs. 38–40). Whether it degenerates after excystment or merely was not demonstrated because of faulty technique, or for other reasons, cannot be decided.

Scattered through the cytoplasm of the active form there are sometimes seen a few very basiphilic granules. These stain even darker with iron-alum-hæmatoxylin than does the nuclear chromatin, and also give the typical reaction with Bonney's triple stain and methyl green. They increase to such an extent in the cyst that during part of that stage they effectually obscure the entire contents of the cell. For reasons which will be discussed later these are termed metachromatic granules.

Seven individuals with two nuclei have been seen. They probably represent cases where fission was prevented, by some unknown reason, from following mitosis.

When the resting condition is entered the flagellates cease movement, lose their flagella, round up into a perfect sphere and secrete an extremely thick wall (Fig. 26). From this early spherical stage they pass into an indefinitely wrinkled condition, which must result from drying of the cell contents (Figs. 28 and 29). A gradual disappearance of the starch bodies can be traced and correlated with the wrinkling of the cyst. Chloro-zinc-iodide and sulphuric acid followed by iodine both indicate the wall to be composed of cellulose.

Life History.

So far as could be determined the life history consists of two phases, (1) active life and (2) rest and reorganization through encystment. During the time of activity *Polytomella* reproduces by means of longitudinal fission. No multiplication whatever takes place during the period of rest. This is well established since hundreds of stained specimens have been examined and many living ones watched continuously during the process of excystment without the slightest evidence of propagation. *There is no indication of a sexual phase in any part of the life cycle.*

Mitosis.—By far the greater number of flagellates in which mitosis has been studied possess within the nucleus an endosome and, surrounding this body, scattered chromatin granules. It is from this scattered material that the chromosomes are usually formed. In Polytomella citri we have a different story, all of the chromatin being located in a single central body, the karyosome [1] (*Parapolytoma satura* has a very similar nucleus, Jameson, 1916).

The orderly behavior of the karyosome during the early part of the prophase is very interesting. The first indication of mitosis is the bisection of this body in a plane transverse to the long axis of the cell-body. The two parts appear to be equal and they pull slightly apart, leaving between them a light cloud. A second division follows the first and forms at right angles with it. The karyosome now consists of four parts which still have the chromatic cloud between them. In the next stage that could be found eight karyosomal bodies, imbedded in the chromatic cloud, were visible. Since the karyosome is spherical we would expect a division, corresponding to the third cleavage of a fertilized egg, between the last two. It may be that such is the case, but the minute size of the objects made it impossible to determine. The division of the karyosome continues until more than twenty particles can be made out, still imbedded in the chromatic cloud. It is interesting to note that the anchoring strands are still persisting. Careful observations were made in an attempt to determine whether they connected, internally, with individual granules or were a part of some achromatic supporting structure within the karyosome, but without results.

In the next step the chromidial cloud lightens, the nucleus becomes transversely elongate to a slight extent, there is a condensation of the numerous small granules and a spireme appears. This consists of seventeen or eighteen chromatin bodies connected by slender achromatic strands. When the nucleus lengthens more and an indication of a spindle appears the chromatin knots on the spireme have been reduced in number to nine and have increased considerably in size. It cannot be said whether this results from the side to side pairing of the eighteen earlier granules or the contraction of alternate connecting strands as evidence was found for both. The achromatic connections still exist between these, the definitive chromosomes. The spireme is now near the equator of the spindle, and in all later stages the strands between the chromosomes have disappeared. The last figure which can be classed as prophase shows the chromosomes lining up on the equator to form a horse shoe around the spindle. The chromosomes which are first freed from each other by the disappearance of the strands are considerably longer than they are broad. The length is quickly reduced, so that when they split at the metaphase the two daughter chromosomes resemble two balls that are in contact. Several very clear polar views of the metaphase were found which indicated the number of chromosomes to be nine. These are arranged in the horseshoe-shape which was noted in side view.

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[1] There is no question concerning the nature of the chromatic body within the nucleus of *Polytomella* and since karyosome has a more restricted meaning than endosome it can well be used here, though I consider that endosome, as suggested by Minchin, is a valuable term for the nucleoli of many protozoan nuclei.

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As noted above a centriole could not be demonstrated in the resting cell. With the elongation of the nucleus and formation of the spindle in the late prophase a granule appears at the poles of the spindle (Figs. 13–15). On the anterior side of the nucleus a line connecting the poles is seen on the nuclear membrane. This, which we shall tentatively term a paradesmose, becomes much more striking in the later stages. Hesitancy in definitely naming this structure is due not to doubt concerning its occurrence, but to the inconclusive demonstrations of a constant centriole, which, presumably, would give rise to the paradesmose.

With the progress of the chromosomes towards the poles the spindle widens to equal the diameter of the horse shoe of chromatin until the band has traversed two thirds of the distance to the poles. Some of the chromosomes now advance ahead of the rest, which show a tendency to clump together. At the same time the nuclear membrane is beginning to constrict at the equator. A little later the dividing nucleus is drawn out into a cylinder with pointed ends, and the chromosomes are clumped into several masses at the ends of the slender cylinder. With the cylindrical shape of the nucleus comes an enlargement of the basal granules, preparatory to their division which will take place just before constriction of the nucleus is complete. At this time the chromatin is clumped into a single mass. The two basal granules divide to form four, each of which gets one of the old flagella. Although no unquestionable figures were found which showed the outgrowth of new flagella it is presumed that this is the method of formation, because if the old flagella split we would expect that to happen at the same time that the basal granules divide and that is not the case. The duplication is by outgrowth in *P. agiles* (Doflein, 1916), *Dunaliella* (Teodoresco, 1905), *Stephanoptera* (Dangeard, 1910), all belonging to this same family.

The history of the chromatin from the beginning of the telophase, when there are single masses at the poles, until the construction of the daughter nuclei is complete is a rapid return through the same stages in reverse order that were passed through in the prophase. The solid masses break up to form a spireme which goes back through the quadri- and bi-partite karyosome to the single condition with which we started. By the time the quadri-partite condition of the karyosome is reached the cell shows marked furrows on both the anterior and posterior ends. In most flagellates fission has been described as beginning at one or the other end. The present case is an example of equal furrowing all around the cell-body. The resting condition is reached by the nuclei, or is very closely approximated, by the time fission is complete. It is interesting to note that the two daughter nuclei are frequently not advanced to the same degree.

We must now return to the first elongation of the nucleus and find what happens to the area of dense cytoplasm which surrounds the nucleus. A glance at the figures from metaphase on will show that there is a fairly even distribution of this material to the daughters. Sometimes it surrounds the nucleus in an even layer, in others it is almost all on one side, and in rare cases it is slightly drawn away from the nucleus. A band of this substance can be seen stretching between the daughter nuclei just before the cells separate. The chromatic nature of this material probably indicates that, although the nuclear membrane remains intact during mitosis, there is some substance of the nature of oxychromatin that is dissolved and passes through the nuclear membrane. This would make the cytoplasmic area under discussion correspond to the archoplasm of Boveri and the archiplasm of Conklin and Wilson. There was no indication of granules of chromatin going bodily through the nuclear membrane.

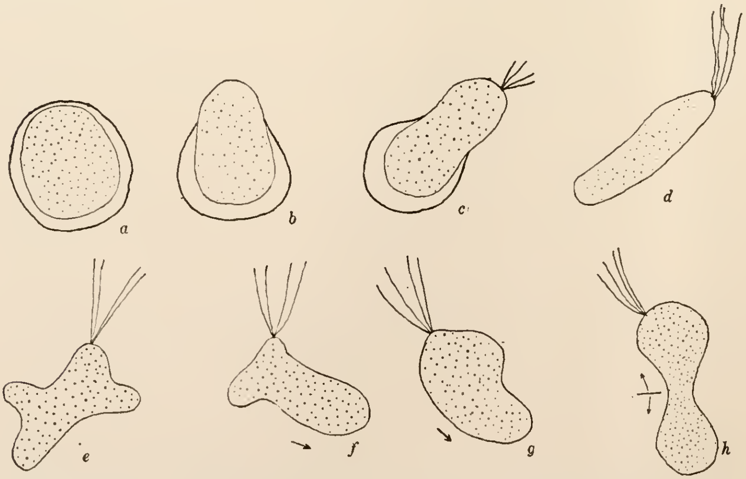
Encystment.—When the cell enters encystment the cellulose wall is evidently formed very rapidly. Only a few individuals were found that did not have a perceptible membrane and in these cases it was only a matter of several hours until they looked quite old and sturdy. While the cyst is still spherical the starch bodies are well under way to disappearance (Figs. 26 and 27). When the wrinkling of the cyst has reached the degree which typically makes the general outline triangular the starch is all gone. Observation of many stained sections of early spherical cysts did not reveal any remnant of the basal granules.

As soon as the organism has become encysted the region immediately surrounding the nucleus becomes filled with spherical bodies which are more basiphilic than the nucleus itself. These bodies increase in extent with the aging of the cyst and soon come to fill the entire cell. In many cases the nucleus, which has now decreased to half its original size and stains very faintly, is obscured. Unless a study of this stage is made very critically one might be led to believe that the nucleus has broken up to form chromidia. Such is not the case. Whenever the knife cut through the nucleus or near it the karyosome, anchoring strands, and nuclear membrane make a perfect miniature, in both size and staining character, of the nucleus of the active form. In the hundreds of such sections examined there was no suggestion of chromatin particles leaving the nucleus. Since this material does not come from the nucleus and stains more deeply with hæmatoxylin, Bonney's triple stain, and methyl green than does chromatin the name metachromatic granules is justified. It is of interest that the increase of metachromatic granules is highly correlated with the disappearance of starch.

After remaining in this condition for some time the granules gradually drain out of the central part of the cell and collect around the periphery, mostly in the angles. The previous spherical form is lost and they assume various shapes. In most cases they entirely disappear before excystment.

When the metachromatic granules are distributed through the entire cell with only small dots of cytoplasm visible, acid dyes, such as Bordeaux Red and eosin, have no effect on what cytoplasm can be seen, even when extra high percentages are used. With the migration of the granules to the periphery the cytoplasm regains its affinity for acid dyes.

After the metachromatic granules have left the immediate vicinity of the nucleus and the cytoplasm has become quite clear we get the first indication of a new centriole and basal granule, in the form of a thickening at the base of one of the anchoring strands. This thickening next appears as a chromatic ball on the strand, a part of which can be seen on each side of the ball. Later it continues to the outside of the nuclear membrane. The basiphilic material that has been drawn out of the karyosome leaves the latter body without disfiguring it. Thus we see that the centriole and basal granules are not formed from a slice of the karyosome, but are taken from that structure by some definitely organized scheme. The body on the outside of the nuclear membrane divides, leaving one portion, supposedly the centriole, while the other portion migrates out through the cytoplasm, spinning a rhizoplast between it and the centriole. Before



Text figure *A*. Diagrammatic representation of stages in excystment and the activity of a newly excysted individual. Arrows indicate direction of movement.

reaching the periphery the single basal granule divides, the rhizoplast dividing with it, to form the two basal granules. The flagella do not develop until the organism is partly excysted.

Excystment is ushered in by the dissolution of the cyst wall on one side (Text Fig. *Aa*). Many stages of this thinning process can be seen before the living cell comes in contact with the outside. The first step in escaping from the cyst seems to be due to the absorption of water, which swells the cell and causes it to protrude through the dissolved opening. Complete freedom from the cyst wall is attained by amœboid movement of the newly formed flagellate. Outgrowth of flagella begins just prior to flowing away from the old wall.

For periods varying from one to fifteen minutes the newly excysted individual shows only amœboid movement, without regard for the polarization caused by the presence of flagella (Text Fig. *Ae, f, g, h*). With the cessation of amœboid movement they assume the typical form and swim off.

Discussion.

Affinities.

The presence of starch within the cell-body of *Polytomella citri* and the typical Phytomonad symmetry are sufficient to establish its position with the order Phytomonadina. Further, the fact that the pellicle divides with the cell at fission places this organism within the family Polyblepharididæ.

This family was suggested by Dangeard in 1887 to include, at that time, *Pyramimonas tetracynchus* Schmarda, *Chloraster gyrans* Ehrenberg, *C. agilis* Kent and *Polyblepharides singularis* Dangeard. In 1905 Teodoresco placed *Dunaliella salina* in this group. This is a marine protozoan, as the name would indicate. Griffith (1909) described *Pyramimonas delicatulus*, taken from a pond in England. In 1910 Dangeard made another contribution to the Polyblepharididæ with his description of *Stephanoptera fabreœ*, a salt water form. This species is one of the largest of the family, reaching 35 micra in length. *Pyramimonas*, 40 micra, being the only one exceeding that size. All of the members of this group possess the ability to change their shape to some extent and *Spermatozopsis exsultans* (Korchikoff, 1913) has the distinction of leading the family in this respect. *Astermonas* was the last holophytic genus described (Artari, 1913).

Dangeard instituted this family to include holophytic, unicellular algæ and all of the species mentioned above would fit in with that scheme. They possess a chloroplast, a pyrenoid for the formation of starch, and a red pigment spot may or may not be present. In *Dunaliella* the green is sometimes obscured by another pigment which gives the organism a vermillion color.

A phylogenetic series might be made, beginning with the six or eight flagellated Polyblepharides, going through the five flagellated *Chloraster*, *Pyramimonas*, which possesses four flagella, *Stephanoptera*, with two flagella, and culminating with the biflagellate *Dunaliella*, the nearest approach to *Chlamydomonas*. With the exception of *Dunaliella* all species have four longitudinal ridges. One might suggest that the scheme could be arranged in the opposite direction, but since the specialization of the Chlamydomonad type is much greater than that of the Polyblepharididæ this order seems the more reasonable.

In 1910 Aragao described *Polytomella agilis* and suggested that ts relationship was with the Protomonads. Doflein (1916) demonstrated the presence of starch in the same species and consequently, reclassified it under the order Phytomonadina, and since its pellicle divides at fission its family is Polyblepharididæ. This colorless form, possessing four flagella, stands in the same relation to *Pyramimonas* that *Polytoma* and *Paraplytoma* do to *Chlamydomonas*. Dangeard's family no longer includes only holophytic algæ, and the green color must be omitted from a characterization of it.

The protozoan dealt with in this paper differs from Aragao's species in the following points: an eye-spot is present in *agilis* while *citri* possesses no such structure; the nucleus of *agilis* contains scattered chromatin while in *citri* it is concentrated in a single karyosome, the four flagella of *agilis* arise from four blepharoplasts and *citri* has only two basal granules. These differences are not sufficient for generic distinction, but justify the formation of a new species.

It is to be regretted that so little study has been made of the finer points of structure of the members of this group, the importance of which must be realized when we consider its position on the threshold of the Phytomonadina.

Life History.

With the exception of *Polytomella agilis* and *Dunaliella salina* the descriptions of the members of this family would lead us to believe that the life history consists of two phases, binary fission during the active stage and rest and reorganization through encystment. Dangeard (1889) describes the cysts of *Polyblepharides* as spherical bodies with a gelatinous wall. This is the only one which has such a covering. To the same author is due the observation that *Pyramimonas* forms cysts with a very tough membrane. Germination of the latter was not observed, but in the former it gave rise to a single organism, which, after exhibiting typical amœboid movement for several minutes would assume the usual shape and swim off by the activity of their flagella. This same observation was made on *Polytomella citri.* Hamburger (1905) noted the formation of cysts by *Dunaliella* and also saw numerous empty shells but did not observe any in the process of excystment. The cysts of *Stephanoptera* are spherical and have a very heavy wall. Dangeard mentions the fact that a few of these had two nuclei, and suggests that there may be some autogamous process during encysted life. The binucleate condition, however, probably does not have any more significance here than it does in the active stage of *Polytomella citri,* where we have interpreted it as representing cases of cytodierisis being prevented from following nuclear division.

Conjugation has been figured by Aragao for *Polytomella agilis* and by Teodoresco for *Dunaliella.* In both cases the figures and description are very incomplete and unconvincing. There are none of the few figures given that could not more easily be interpreted as stages in fission than as representing conjugation. This is especially true for the work of Aragao. In spite of the fact that sexual reproduction probably plays no part in the life of most of the members of this family, we would not be surprised to find sexual processes in *Dunaliella* because of its near relationship to *Chlamydomonas.* However, until a more adequate study has been made with this problem in view it is well to be hesitant in accepting the Polyblepharididæ as the group which gave rise to the sexual phenomena which are so well developed in the two higher families of the Phytomonadina.

Mitosis.—The nucleus of most flagellates contains, in addition to the endosome, scattered chromatin granules which seem to be suspended on a linin net-work. The rôle of the endosome in nuclear division is generally one of two types. The first, typified by the Euglenoids, is the case where this body is drawn out into a dumbbell shape, in the interior of the spindle, with the two knobs forming polar caps. With the progress of the chromosomes to the poles this structure is severed at the equator and forms the endosomes of the daughter nuclei. Some writers (Belar, 1916*A*, Berliner, 1909, Schussler, 1917) have termed this central body of the Euglenoid a "centrocaryosome," assigning to it the function of a centrosome. Hall, however, has questioned this view. Another type of behavior is illustrated by the endosome of *Polytoma* (Entz, 1918) and *Chlorogonium* (Hartmann, 1916) which disappears during the prophase. Aragao described the formation of two sets of chromosomes in *Polytomella agilis*, one from the scattered chromatin, the other from the endosome. Doflein (1916), repeating his work, found that the endosome disappeared during the prophase without contributing to the formation of the chromosomes.

It is very evident that a comparison cannot be made of the mitotic phenomena of *P. citri* and those of any form just mentioned, not even the other species of its own genus. However, a similar form is found in *Parapolytoma satura* (Jameson, 1914) which has all of the chromatin gathered into a single body, the karyosome, which is suspended from the nuclear membrane in much the same way as in *P. citri*. Unfortunately Jameson's account begins rather late in the prophase, his first figure showing the karyosome broken into a number of bodies which are connected by linin strands. He states that the greatest number of these ever found is sixteen or eighteen, which fuse in two's or three's to form the eight definitive chromosomes. The same behavior has been found in *P. citri*, with the addition of the early stages in the fragmentation of the karyosome. Although the chromosomes of *P. agilis* are formed from scattered chromatin the five definitive ones arise in much the same way, by the pairing of ten chromatin granules which form a ring around the endosome. Doflein analogizes this fusion with the conjugation of homologous pairs of chromosomes in metazoan and metaphytan cells.

Thus we see that the greatest similarity in the activity of two cell-organs is not to be found in two organisms whose general structure is most nearly alike, but in two that have the nearest approach to identity in that particular organ.

Encystment.—There is a remarkable correspondence of the encysted forms of the various species of this family. *Pyramimonas delicatulus* and *Spermatozopsis exsultans* are the only ones in which cysts have not been described. There is only one case where division takes place within the cyst (Doflein, 1916). Aragao did not find division in this same species. This author met with the same difficulty that was encountered in the present work, namely, that the cyst could not be stained. This fact is probably responsible for the fragmentary study of this important phase of the life history of the Polyblepharididæ, as no adequate account has heretofore appeared.

In *Polyblepharides* and *Polytomella citri* the living organisms have been watched during excystment and in both cases amœboid movement was observed for some minutes before any use was made of the developing flagella. *Dunaliella* and *Polytomella citri*, and probably all of their near relatives, leave the cyst wall behind without dissolving it. The latter dissolves on one side in order to gain freedom, but the remainder of the shell stays in the culture for some time.

Since the pioneer work of Guillermond and Meyer metachromatic granules have been described from bacteria to metazoa. The fact that these granules not only increase greatly during the early days of encystment, but also are dissolved, not thrown off, before excystment indicates that they have some important rôle to play in the life of *Polytomella citri*.

Centriole and Basal Granules.

The observations made on this subject in the present work are as follows: There are two basal granules from which arise four flagella. These granules divide during the telophase of mitosis.

The poles of the spindle are lodged in centrioles which are located on the outside of the nuclear membrane. An indication of rhizoplasts connecting the basal granules with the nucleus have been seen in both resting and dividing cells. In the early cysts no basal granules can be seen. Before excystment a centriole and new basal granules arise from the karyosome. This material is drawn out of the latter body without disfiguring it. There is, at first, a single basal granule which divides before reaching the periphery. A rhizoplast is very evident during this phase. The new flagella grow out after the organism is partly excysted. The questions not answered are: is a centriole present in the resting cell of the active form; what becomes of the rhizoplasts that are so evident before excystment; are the basal granules drawn into the karyosome at the beginning of encystment or do they degenerate?

In view of the fact that a centriole can be seen in division stages, is very pronounced in the cysts and that Doflein has demonstrated an extra-nuclear centriole in *Polytomella agilis* I think we can conclude that such a center is present in the interkinetic cell of *P. citri*. In addition the paradesmose, which is quite evident, would, presumably, come from a centriole. Jameson (1914) says that *Parapolytoma* has no centriole. He mentions *Chilomonas* and *Polytoma* as other examples of flagellates without this division center. It is to be noted that Belar (1916) demonstrated a centriole in *Chilomonas* and Entz (1918) showed that *Polytoma* has a very definite one on the inside of the nuclear membrane, with a heavy centrodesmose appearing at division.

Aragao figures rhizoplasts connecting the basal granules of *P. agilis* with the nucleus. Hamburger did likewise for *Dunaliella*. In view of these observations, coupled with what has been seen in *P. citri* I believe we are justified in assuming that the rhizoplasts are constant structures in *P. citri*.

Berliner states that the basal granules of *Copromonas* are drawn into the endosome at division. His figures, however, are not at all complete. Jameson finds that the basal granules of *Parapolytoma* never divide, but three of the four individuals resulting from division receive new basal granules from the karyosome. Entz finds that the centriole and blepharoplasts of *Polytoma* arise in the same way as in *P. citri* with the exception that the centriole remains inside the nuclear membrane. Although it is impossible to definitely answer the third question suggested above, it seems more probable that the granules degenerate because if they entered the karyosome we would expect them to maintain their integrity to sufficient extent to prevent the karyosome from appearing perfectly homogeneous. Such is not the case.

Summary.

The flagellate herein described belongs to the genus *Polytomella* (Aragao, 1910). The specific name "*citri*" is proposed. The size ranges from 10 by 7 micra to 14 by 10 micra. Outside of the absence of chlorophyll the structure is typical for the family Polyblepharididæ. Although it is a colorless, saprophytic organism the cell-body contains numerous starch bodies.

The life history consists of active stage, in which reproduction is by binary fission, and encystment. No reproduction takes place during encystment. There is no indication of any sexual process at any point of the life cycle.

Conjugation is not well established in any species of the family.

The chromosomes arise as a result of the fusion of chromatin particles which have come from the karyosome.

The cysts are filled with metachromatic granules. They usually disappear before excystment.

A new centriole and basal granules are given off from the karyosome previous to excystment. Flagella do not grow out until the cell comes in contact with the outside. Amœboid movement is the means of locomotion for several minutes following excystment.

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EXPLANATION OF PLATES.

Figures 1 and 25 to 29 made from living material. Figures 2 to 24 made from material fixed in hot Schaudinn's fluid and stained in hot iron-alum-hæmatoxylin, counterstained with Bordeaux red. Figures 30 to 41 were made from specimens fixed in Bouin's, stained in hot iron-alum-hæmatoxylin and counterstained with either Bordeaux red or eosin. All drawings made with Abbe camera lucida. Magnification 2500 X.

Plate I.

Fig. 1. Diagrammatic camera lucida drawing of living individual pressed between two mold hyphæ. *cv*, contractile vacuole; *s*, starch bodies.

Figs. 2, 3 and 4. Showing extreme changes of shape.

Fig. 5. Binucleate individual.

Fig. 6. Resting nucleus. Extensive area of slightly chromatic cytoplasm surrounding nucleus and filling posterior end.

Figs. 7 to 24. Stages in the division of the nucleus and cell-body.

Fig. 7. The transverse splitting of the karyosome, the first indication of mitosis.

Figs. 8 to 11. Continued fragmentation of the karyosome resulting in over twenty chromatin particles imbedded in a chromatic cloud. Anchoring strands persisting.

Fig. 12. Nucleus becomes slightly elongate, anchoring strands disappear, and seventeen or eighteen chromatin particles connected by slender achromatic strands become visible.

Fig. 13. The chromatin particles of the preceding one have paired to produce the nine definitive chromosomes. An indication of a spindle appears and the poles are lodged in centrioles which are connected by a paradesmose on the anterior side of the nucleus.

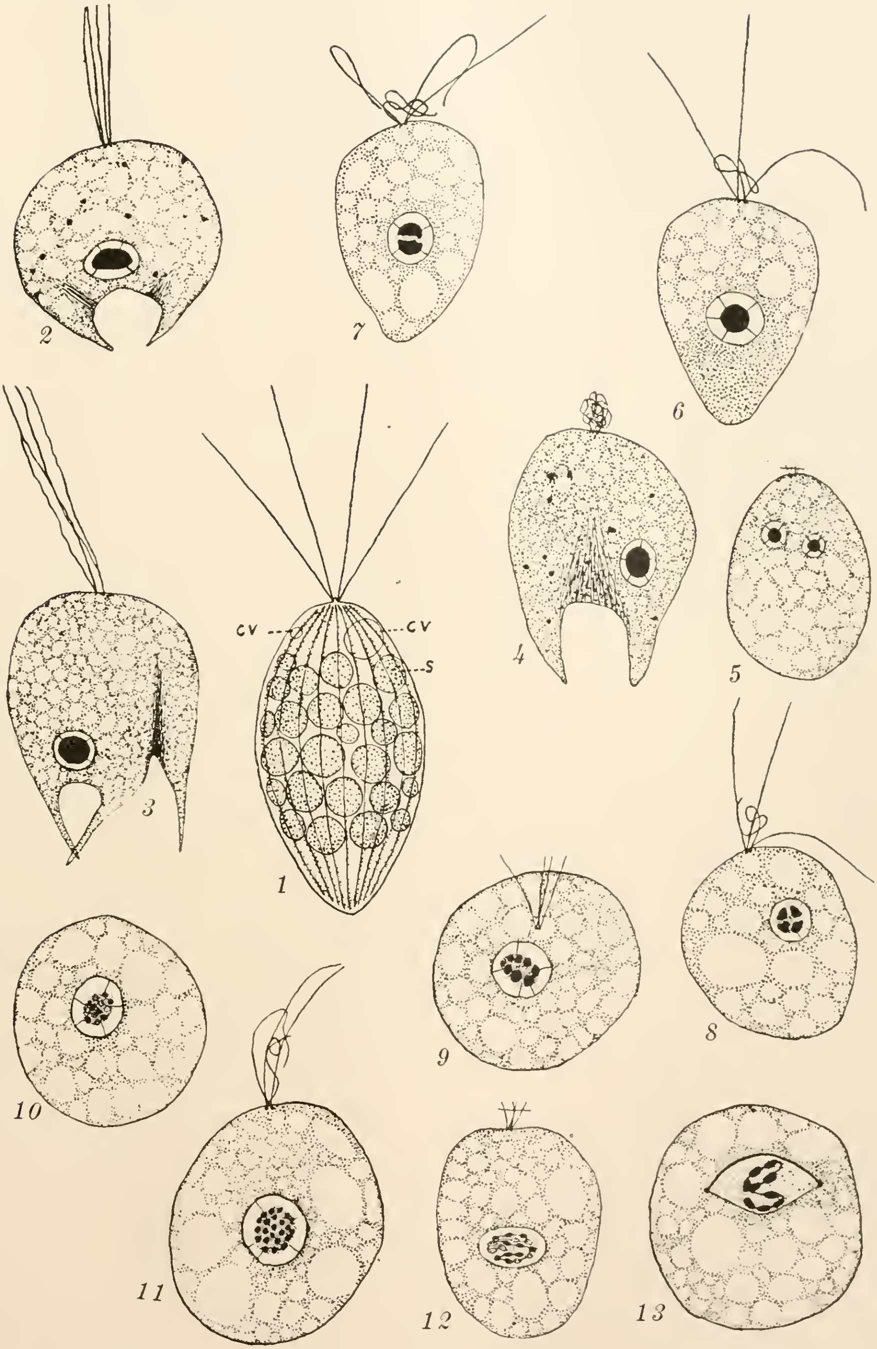
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PLATE II.

FIG. 14. Chromosomes lining up on equator to form horse shoe around the spindle. Paradesmose visible above spindle.

FIG. 15. Metaphase.

FIG. 16. Polar view of metaphase.

FIGS. 17 AND 18. Migration of chromosomes to poles of spindle.

FIG. 19. The nucleus has become elongated into a cylinder with pointed ends and the chromosomes are fusing together. The paradesmose is seen on anterior side of nuclear membrane and the basal granules are enlarging.

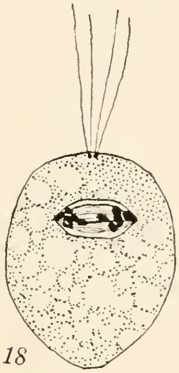
FIG. 20. The chromosomes have fused into a single mass at the poles, and the basal granules have divided.

FIG. 21. The chromatin masses of the preceding one have broken up to form a spireme comparable to that of Fig. 13.

FIGS. 22 AND 23. Reconstitution of the daughter nuclei and division of the cell.

FIG. 24. A case where fission was either delayed or prevented by some unknown cause.

FIG. 25. Beginning of encystment. The flagella have been lost and the cell is packed with starch bodies.



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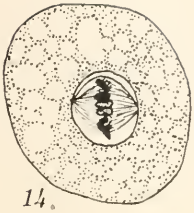

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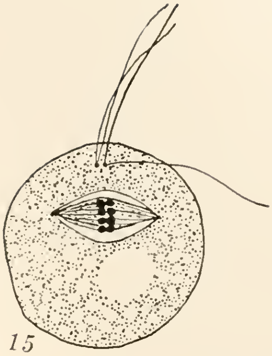

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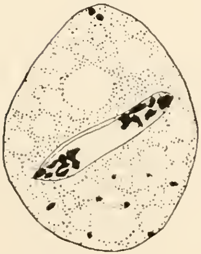

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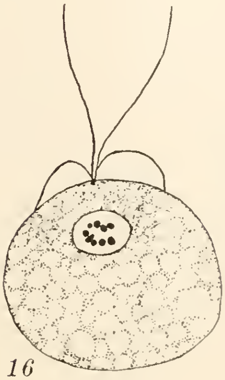

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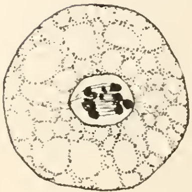

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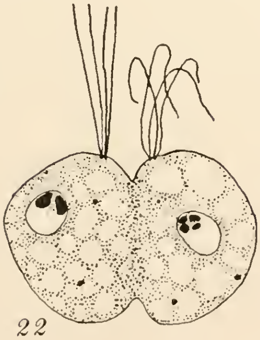

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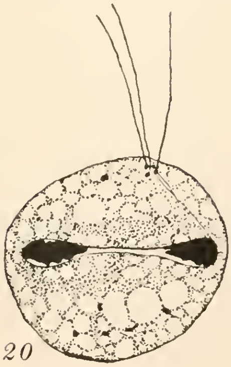

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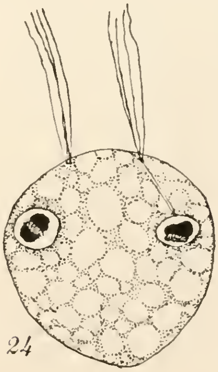

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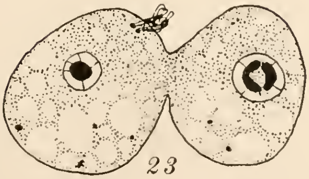

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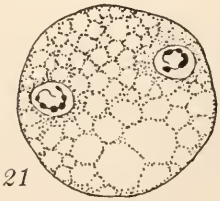

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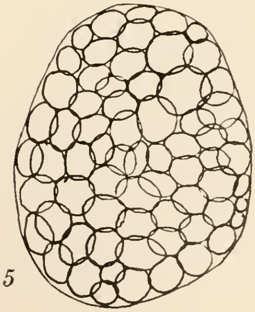

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Plate III.

Figs. 26 to 29. Stages in the formation of wrinkled cyst wall and the disappearance of the starch bodies.

Figs. 30 to 40. Made from sections four micra thick.

Fig. 30. Early cyst. Metachromatic granules are forming around the nucleus.

Fig. 31. The wall has become thicker than in the preceding case and the metachromatic granules are scattered through the entire cell. Nucleus obscured.

Fig. 32. The metachromatic granules are evenly distributed through the cytoplasm. Wall becoming wrinkled.

Fig. 33. The metachromatic granules are migrating to the periphery where they will be dissolved.

Fig. 34. The first indication of budding of the karyosome. A thickening appears at the base of one of the anchoring strands.

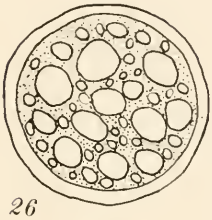
Fig. 35. The material which is going to form the new centriole and basal granules appears as a chromatic ball on one of the anchoring strands.

Fig. 36. The chromatic ball has reached the outside of the nuclear membrane.

Figs. 37, 38 and 39. Stages in the migration of the basal granule towards the periphery.

Fig. 40. The basal granule has divided, the rhizoplast dividing with it.

Fig. 41. Drawing made from whole mount. The organism is partly excysted and the flagella are growing out.



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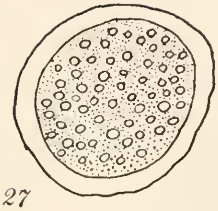

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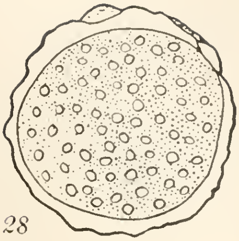

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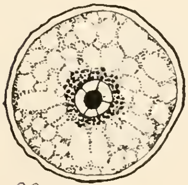

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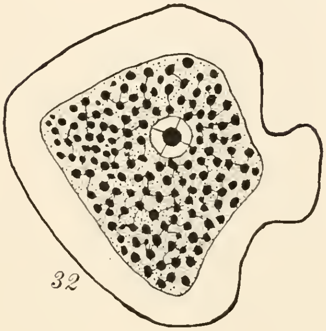

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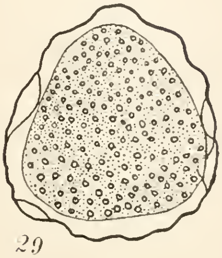

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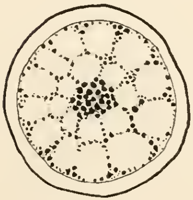

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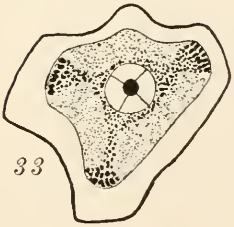

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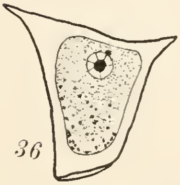

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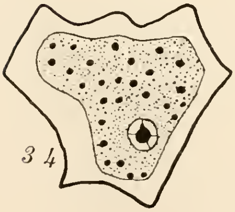

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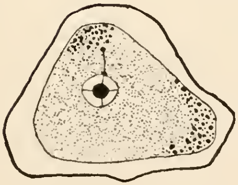

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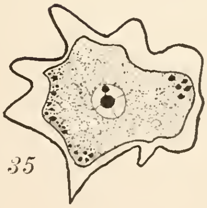

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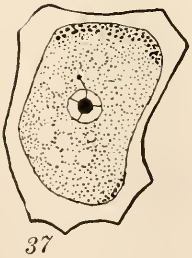

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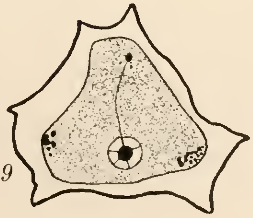

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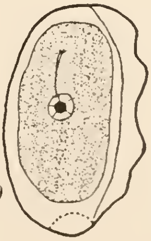

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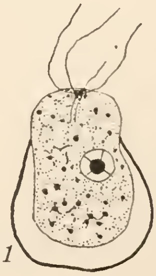

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J. MCA. KATER.

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BIOLOGICAL BULLETIN

TRICHODINA STEINII (C. AND L.) FROM *PLANARIA POLYCHORA* (O. SCHM.).

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In the spring of 1924 the junior author secured specimens of *Planaria polychora* O. Schm. from a pool several hundred yards west of the Biological Laboratory of the University of Virginia. The *Planaria*, in some cases, carried upon their surfaces specimens of *Trichodina Steinii.* These specimens afforded us an excellent opportunity to study the habits and anatomy of this species of large ectozoic peritrichous ciliate. Our observations led us to recognize two interesting facts concerning this species first described by Claparede and Lachmann (58).

The genus *Trichodina* is so closely related to *Vorticella* that it might be placed in the subfamily Vorticellidæ. Indeed Fulton (23) said of the subfamily Urceolariidæ, to which Minchin assigned *Trichodina*, "It is a matter of personal preference whether one considers the Urceolariidæ a subfamily of the Vorticellidæ . . . or as a separate family." With this close systematic relationship in mind, certain homologies between the anatomical details of *Trichodina* and those of *Vorticella* are of interest. The peristome and nucleus complex are clearly the homologues of the peristome and nucleus complex of *Vorticella.* In the free swimming *Trichodina Steinii*, the peristome remains closed (Fig. 3) in a manner that causes it to greatly resemble the closed peristome of a contracted *Vorticella.* A free swimming *Vorticella* has a posterior zone of cilia (Fig. 4). The homologue of these adoral cilia become fused at their bases to form the "velum" of *Trichodina. Trichodina Steinii*, in correlation with its habit as an ectozoön, has developed a horny flexible ring

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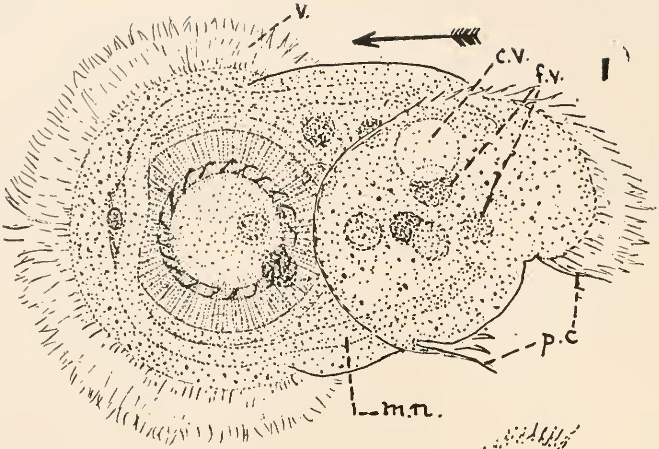

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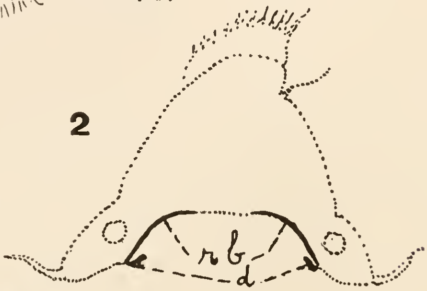

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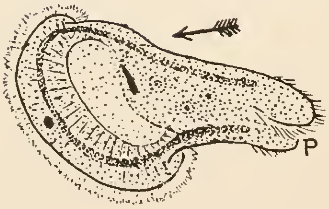

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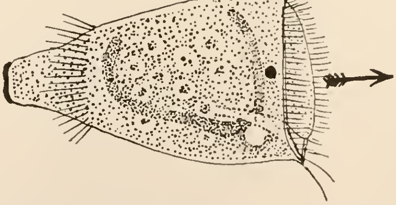

FIG. 1. Dorsal aspect of specimen as seen when fixed to or creeping over host. *v*, velum; *c.v.*, contractile vacuole; *f.v.*, food vacuoles; *p.c.*, cilia of peristome; *m.n.*, meganucleus (U-shaped). Observe the small micronucleus lying at outer margin of the U-shaped contour of meganucleus. × 1000.

FIG. 2. Transverse optical section through diameter of striated horny ring or ring band of adhesive organ. *r.b.*, horny ring or striated ring band; *d*, denticles or hooks. × 1000.

FIG. 3. Free swimming *Trichodina Steinii*. *p*, peristome closed. Note position of micronucleus and meganucleus with reference to the animal's swimming posteriorly, as indicated by the arrow.

FIG. 4. Free swimming form of *Vorticella*. Note position of micronucleus and meganucleus with reference to the animal's swimming anteriorly, as indicated by arrow.

(ring band) as a conspicuous feature of its "adhesive organ." In this complex adhesive organ, with its velum, Louis Agassiz (50) saw homologies between it and the details of an hydromedusa. Because of this he looked upon the *Trichodina* as being the medusa of *Hydra*. Attempting to establish analogies may, therefore, lead one into strange ventures. But a further homology may be seen in the horny ring of the adhesive organ of *Trichodina Steinii*. This ring is of a chitinous texture and lies at the posterior extremity of the body of *Trichodina*. Fauré-Fremiet (05) studied the adhesive substance that *Vorticella* elaborates when it fixes itself to a surface. He found that this adhesive material was a "sécrétion chitineuse." If now the reader will look at our figures 3 and 4, he will see that in the free swimming *Vorticella* and *Trichodina* at the posterior end of each of these ciliates is a region concerned with the secretion of a horny material. In *Vorticella* the horny substance is the adhesive material, whereas the homologue of this horny substance becomes the horny ring or "striated ring band" of the adhesive mechanism in *Trichodinii Steinii*.

Perhaps a more interesting comparison may be drawn between *Vorticella* and *Trichodina* than that based upon homologies. This contrast presents itself in the polarity of the two protozoa. *Vorticella* has its anterior-posterior polarity normal. When a *Vorticella* moves about it swims with its peristome forward as indicated by the arrow in Fig. 4. The careful studies of Jennings (04) indicate that it is a matter of great importance to an animal like *Paramœcium* that its peristome lies towards the line of travel. To reverse this attitude would appear, therefore, to be a radical change. This, however, is what has taken place in *Trichodina Steinii*. These ciliates become quite active when their host is placed under the compound microscope's light and creep about over the surface of the host freely. In all cases they advance posteriorly as they cling lightly to the surface of the host with their adhesive organs. If the disturbance be continued long enough, they will leave the host and swim off into the water. Even the free swimming specimens travel *posteriorly*. When thus traveling, they have the peristome closed and inactive while the velum and horny ring are expanded (Fig. 3). There is thus a complete reversal of polarity of this animal both when it creeps over a surface and when it swims. This reversal of polarity has been overlooked by the earlier observers of *Trichodina Steinii.* Kent (81) implies that *Trichodina pediculus* (Ehr.) has the usual polarity. Fulton (1923) in making a thorough review of the literature of this genus and in his own observations, seems not to have encountered this reversal of physiological and morphological polarity. This author, as well as earlier investigators, has also overlooked the homologies that we have drawn between this ectozoic peritrich and *Vorticella.*

This animal should, therefore, serve as the basis of some interesting work done upon its physiological axial gradient. From what we have seen of its morphology we expect to find that its axial gradients have been reversed in their sequence; for it is seen that there has been a morphological reversal, despite the homologies that we have indicated. In *Vorticella* there is an emphasis placed upon the anterior region in that the loop of the meganucleus and the micronucleus lie in that region. In *Trichodina Steinii* the morphological emphasis is reversed; for in this form the loop of the meganucleus and the micronucleus lie in the posterior region of the cell.

Thus it appears that in *Trichodina* rather complex adaptive structures are but the homologues of simpler structures found in *Vorticella;* but that correlated with the ectozoic mode of living *Trichodina* has undergone a reversal of polarity that involves both its physiological axis and its nuclear complex.

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STUDIES IN ARTIFICIAL PARTHENOGENESIS.

V. The Anomalous Action of Mercuric Chloride.

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Traube[1] in 1909 made the claim that substances of low surface tension were more effective in producing parthenogenesis than those of higher surface tension. In 1913 and again in 1915, Heilbrunn[2] proposed a theory of membrane elevation in the sea-urchin egg, according to which the lifting off of the membrane (in this egg) is the direct result of a lowering of its surface tension. If this surface tension theory of membrane elevation is correct, only those substances which cause a decrease in surface tension can produce typical membrane elevation. So far as previous experiments have gone, this is true. There is no case in which membrane elevation has been produced by an agent which does not sharply lower surface tension (Heilbrunn, '24).

During the past summer an apparent exception was discovered. In the course of some work which we were doing with dilute solutions of mercuric chloride in sea-water, my assistant Mr. D. E. S. Brown called my attention to what was apparently true membrane elevation in the egg of the sea-urchin *Arbacia*. Beautiful wide membranes arose from the egg surface. That these were truly elevated membranes (not swollen membranes) was shown by the fact that they could be made to collapse on the addition of egg albumin to the sea-water.

The parthenogenetic action of mercuric chloride was known to F. R. Lillie and he mentions it briefly in a paper published in 1921[3] (see p. 140).

It seems certain that mercuric chloride as such could have no great effect on surface tension. Apparently, therefore, the fact that the mercury salt causes membrane elevation can not be made to fit in with the surface tension theory. Because of this apparent conflict, it was thought worth while to further investigate the action of the mercuric chloride, with a view to the possible abandonment of the surface tension theory. Although many experiments were performed, only a few of them will be considered and these rather hastily. They suffice to show that the action of mercuric chloride is not a simple one.

If an *m*/10 solution of mercuric chloride is diluted a thousand times with sea-water, then an "*m*/10,000 solution of mercuric chloride in sea-water" is obtained. A solution of this sort is very favorable to membrane elevation. Solutions decidedly more concentrated than *m*/10,000 do not act as well. In an *m*/1000 solution, the membranes, if they lift off at all, become only partially elevated, apparently they stick to several points of the egg surface as they attempt to lift away from it. On the other hand extreme dilutions are also ineffective, although it is possible to obtain good membrane elevation in solutions as weak as *m*/100,000. Thus there is a wide range of concentration in which the mercuric chloride is effective. Most of the experiments were made with an *m*/10,000 solution (in sea-water).

On exposure to the mercuric chloride, membrane elevation does not occur immediately. After 3 or 4 or 5 minutes, the membranes can be seen rising from the surface of the eggs. The effect of the sublimate is not solely a cortical effect. After eggs have been exposed for about 12 minutes other changes may begin to appear. In many cases the cell undergoes what is apparently a cell division. At any rate it becomes constricted. Usually this constriction divides the egg unequally, a large cell and a small cell are formed. In such cases of unequal division the smaller cell usually contains a mass of pigment and the larger cell is comparatively colorless. Now and then instead of an unequal division there is a furrow around the very center of the egg with an accumulation of pigment granules underneath the furrow. No attempt was made to discover whether nuclear changes accompanied the changes in the cytoplasm. If the constrictions of the cytoplasm do represent anything at all comparable to a normal cell division, it is surprising that they occur so soon after the eggs are exposed to the mercuric chloride. Typically the Arbacia egg does not divide until 50 or 60 minutes have elapsed after fertilization. The cytoplasmic constrictions just described make their appearance 12 or 15 minutes after the eggs are placed in the mercury solution. The constriction of the cytoplasm is not followed by anything approaching normal development, even if the egg is removed from the solution of mercuric chloride and placed in normal sea-water.

Ordinarily when Arbacia eggs are exposed to an *m*/10,000 solution of mercuric chloride in sea-water, only about 10 per cent. of the eggs undergo membrane elevation. Why 10 per cent. of the eggs should throw off perfect membranes and the other 90 per cent. should show no change at all, was for a time a mystery.

After a number of experiments under diverse conditions, it was found that the percentage of eggs undergoing membrane elevation could be greatly increased by allowing the eggs to age. If eggs are taken from the ovary and placed directly into dilute solutions of mercuric chloride, no membrane elevation occurs. On the other hand if they are allowed to lie in sea-water in shallow dishes for 5 or 6 hours, practically a hundred per cent. will undergo membrane elevation when exposed to the mercuric chloride. In working with this reagent it is therefore advisable to allow eggs to age before starting to experiment with them.

The effect of aging is shown in the following table. The first column of this table gives the number of minutes the eggs were allowed to remain in sea-water before they were treated with *m*/10.000 mercuric chloride. Only the eggs of a single female were used but these were very plentiful. On removal from the ovary they were placed in 50 cc. of sea-water in a fingerbowl. After a time, as a result of the constant removal of material, the eggs at the bottom of the fingerbowl were no longer evenly distributed, some being more closely clustered than others. In the denser masses of eggs, the aging process apparently proceeded at a slower rate and this accounts for the irregularities in the table. The second column shows the percentage of eggs undergoing membrane elevation when subjected to mercuric chloride. To obtain this percentage two hundred eggs were counted in every instance except in the case of the eggs aged for 268 minutes. Only a hundred of these were counted.

Time Eggs Remained in Sea-water before Treatment with $HgCl_2$, Minutes.	Percentage of Eggs Showing Membrane Elevation.
0	0
5	6½
10	7½
15	10
20	10
30	11½
40	13
50	14½
60	17
81	17½
103	17½
128	15½
158	16
193	18½
238	30½
268	21
300	62½
345	94½

Although the table is not as regular as might be wished, it is clear that as the eggs lie in sea-water they become increasingly susceptible to mercuric chloride. This fact is interesting for at least two reasons. In the first place it apparently does not harmonize very well with the fertilizin theory of fertilization as developed by F. R. Lillie.[3] Aging of eggs, according to this theory, involves a loss of fertilizin, a material which is regarded as essential to fertilization, and yet in the experiment described above, such aging favored a cortical change characteristic of fertilization. But the case of mercuric chloride is certainly a special one, and it is doubtful if any general conclusions for or against the fertilizin theory can be derived from it. This will be more obvious from the later discussion.

Our main interest is elsewhere. Why do eggs after standing in sea-water respond more readily to treatment with mercuric chloride? Possibly in or around eggs fresh from the ovary there is some substance present which exerts an interference. This substance escapes on standing; it may be gaseous. On a priori grounds one would suspect carbon dioxide.

Experimental evidence favors this conjecture. When eggs were allowed to stand until they became highly susceptible to mercuric chloride and were then treated with a solution of the sublimate in sea-water which contained a trace of hydrochloric acid, they did not lift off membranes. If now the solution of mercuric chloride in acidified sea-water was shaken vigorously for a few minutes it again became potent as a stimulant to membrane elevation. In one experiment 4 cc. of $n/10$ HCl was added to 200 cc. of sea-water. To this was added 11 cc. of $m/1000$ $HgCl_2$. The resultant solution had a p_H below 6.8 and caused no membrane elevation. It was shaken until the p_H rose to above 7, but it still remained ineffective. It was then shaken vigorously for several minutes, and the p_H rose well above 7. After such shaking it produced membrane elevation generally. The effect of the shaking is to remove carbon dioxide from the solution. It seems certain from this experiment that the presence of carbon dioxide interferes with the production of membrane elevation by mercuric chloride.

Our first point then is the fact that mercuric chloride causes membrane elevation only in the absence of any considerable quantity of carbon dioxide.

A second point, which was not investigated as closely as it might have been, is the fact that sublimate treated eggs, if centrifuged one or two minutes after the exposure begins, do not lift off membranes. In one experiment no membranes were lifted off when eggs were centrifuged either one or two minutes after they had been placed in a $m/10{,}000$ $HgCl_2$ solution. Those centrifuged $3\frac{1}{2}$ minutes after being placed in the solution showed membrane elevation in 91 out of 100 cases. The centrifugal force used was 4,968 times gravity and in this experiment the eggs were centrifuged for 40 seconds in each instance. Apparently it is not necessary to centrifuge the eggs for as long a time as 40 seconds. In other experiments it was found that centrifugal treatment for only 5 seconds was enough to prevent all but a small percentage of the eggs from undergoing membrane elevation. The reason for the effect of the centrifuge is not certain. A plausible hypothesis, but by no means the only one, is that the mercuric chloride reacts with the jelly of the egg to form mercurous chloride and chlorine, and that it is the chlorine which is important for membrane elevation. Centrifuging removes the jelly from proximity to the egg surface. In favor of this view is the fact that mercuric chloride regularly does break down into calomel (or mercuric oxychloride) and chlorine in the presence of organic substances (Mellor,[4] Gmelin-Kraut [5]). On the other hand it should be pointed out that if the jelly is removed from the eggs either by shaking or centrifuging or both, and the eggs are then subjected to mercuric chloride solution, they undergo membrane elevation just as well as when the jelly is present. This fact need not interfere with our hypothesis. We can assume that although ordinarily the mercuric chloride reacts with the jelly to form chlorine, in the absence of chlorine it may react with the cortex of the egg itself.

A test of this point might be made by removing the jelly, treating with mercuric chloride and then centrifuging. If the above interpretation is correct then centrifuging of jelly-free eggs should not interfere with membrane elevation produced by the mercury salt. Unfortunately such a test was not made.

But there is other evidence in favor of the view that mercuric chloride reacts with some part of the egg. Sublimate solutions lose their effectiveness when allowed to stand over eggs. In one instance, after an $m/10,000$ solution had stood over eggs for 10 hours it in large measure lost its effectiveness as a stimulant to membrane elevation. Although a control of the same solution produced typical membrane elevation, the solution which had stood over eggs caused at best only a partial and slight membrane elevation. Even in the absence of eggs an $m/10,000$ mercuric chloride solution slowly loses its power to cause membrane elevation. The loss of this power is much more rapid in the presence of eggs.

If we assume that mercuric chloride gives off chlorine and it is this substance which causes membrane elevation, it should be possible to duplicate the action of the mercury salt with chlorine itself.

Chlorine gas was manufactured by adding hydrochloric acid to potassium permanganate and the gas was then passed through sea-water. If two drops of the solution of chlorine in sea-water are added to 20 cc. of sea-water the resultant solution is then effective in inducing membrane elevation. The results with chlorine itself are not as good as those with mercuric chloride. It is hard to regulate the amount of chlorine and only a very narrow range of concentrations may be used. One drop more or less of chlorine water added to 20 cc. of sea-water would often determine whether the resultant solution would be effective or not. Moreover the number of eggs introduced into the chlorine solution was an important factor. Thus in one experiment two stender dishes were each filled with 20 cc. of sea-water plus 2 drops of chlorine water. Many eggs were placed in one of the dishes, only a few in the other. In the latter case 78 per cent. of the eggs underwent partial or complete membrane elevation; in the dish containing many eggs no membrane elevation occurred at all.

It is easy to suppose that the addition of mercuric chloride to eggs furnishes a mechanism for supplying approximately the proper dose of chlorine to the egg surface. This is probably the reason that solutions of chlorine alone are not as effective as the mercuric chloride.

An argument that might be advanced against the idea that the action of mercuric chloride is due to chlorine is the fact that mercuric nitrate is also effective in causing membrane elevation. In order to exclude the presence of chlorine, the mercuric nitrate was made up in a 0.9 *m* sugar solution. A solution roughly *m*/10,000 was prepared. The action of such a solution is very peculiar. Extremely wide membranes are lifted off, membranes which are quite different in appearance from the normal-looking ones produced by mercuric chloride. It would be easy to show that a reaction occurs between the sugar and the mercuric nitrate, but no effort was made to determine the products of such a reaction. However it is believed that one of the reaction products causes the membrane elevation.

From the evidence cited it is obvious that the action of mercuric chloride is complex. It appears certain that the reagent does not act in its original form but undergoes some sort of a chemical transformation. There is good reason to believe that chlorine is produced, and that this substance acts on the eggs and incites membrane elevation.

If this view is correct then the fact that mercuric chloride solutions are effective in causing membrane elevation can not be used as an argument against the surface tension theory. If the sublimate forms chlorine we know that a substance of extremely low surface tension must be present.

It is possible that many more facts with regard to the action of mercuric chloride on egg cells or cells in general might easily be obtained by a further study of the sea-urchin egg in the presence of this reagent. Our only interest in the subject lay in the possibility of overthrowing the surface tension theory of membrane elevation. As soon as it was evident that no such overthrow was possible on the basis of the mercury evidence, no additional experiments were planned.

Summary.

1. Dilute solutions of mercuric chloride in sea-water cause typical membrane elevation in the sea-urchin egg in spite of the fact that they presumably do not lower surface tension.

2. The action of the mercuric chloride is favored by aging the eggs. Eggs fresh from the ovary are not acted upon, and the percentage of membrane elevation on treatment with the sublimate solution increases in proportion to the time the eggs have stood in sea-water before being subjected to the reagent.

3. The favorable effect of aging is apparently due to the removal of carbon dioxide. The addition of carbon dioxide prevents membrane elevation by mercuric chloride.

4. If eggs are centrifuged one or two minutes after the treatment with mercuric chloride is begun, membrane elevation is generally prevented.

5. Solutions of mercuric chloride in contact with eggs lose their power of provoking membrane elevation.

6. The facts cited in 4 and 5 are regarded as evidence in favor of the view that mercuric chloride reacts with the jelly or cortex of the egg to form chlorine. Such a reaction is in accord with the usual behavior of mercuric chloride in the presence of organic materials.

7. Chlorine gas is effective in producing membrane elevation.

8. The action of mercuric chloride in causing membrane eleva-

tion is probably due to the formation of chlorine. Since chlorine has a very low surface tension, the fact that mercuric chloride causes membrane elevation can not be used as an argument against the surface tension theory.

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THE SPERMATHECA OF *EURYCEA BISLINEATA*.[1]

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Since 1785 it has been known, first through Spallanzani, that fertilization in salamanders is internal. Later there were found at intervals in several species "receptacula seminis" into which spermatozoa are received and stored. So far as is known in most species of salamanders the spermatozoa are transferred some time before egg-laying to the cloaca of the female by means of spermatophores deposited by the male and received by the female either actively or passively, the method of deposition and reception varying somewhat in the few species which have been noted at breeding.

The "receptacula seminis" or spermathecæ[2] of several forms has been noted by Rathke (1820), Leydig ('53), Siebold ('58), Jordan, Fisher and Stieda ('91), and Kingsbury ('95). The work of Dr. Kingsbury is especially important as a comparative study of the spermathecæ of *Necturus maculatus*, *Diemyctylus viridescens*, *Desmognathus fuscus*, *Amblystoma punctatum*, *Plethodon glutinosus* and *Spelerpes bislineatus* (*Eurycea bislineata*). In all of these forms the spermatheca is dorsal to the cloaca and consists of a number of tubules. These tubules open severally into the dorsal wall in *Necturus*, *Diemyctylus*, and *Ambystoma* and cover comparatively large areas in this region. In *Desmognathus*, *Plethodon* and *Eurycea* the organ is more compact and opens by a single central tubule into the cloaca.

Kingsbury describes but one specimen of *Eurycea bislineata* taken in October and containing no spermatozoa. It is the purpose of this paper to continue the work on *Eurycea*, describing the mature spermatheca at various seasons and in the course of its development.

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[1] Contributions from the department of Zoölogy, Smith College, No. 131.

[2] Dr. H. H. Wilder calls attention to the fact that the correct word is Spermatotheca, using the root of the word (spermato-) instead of the nominative stem.

I.

The Mature Spermatheca.

The spermatheca of *Eurycea bislineata* is a compact, definitely bounded organ. Lying in a median dorsal position with reference to the cloaca it extends from the opening of the organ, a point cephalad to the vent of the cloaca, to the posterior wall which lies above the vent of the cloaca. A mature spermatheca is usually from 1/2 mm. to 2/3 mm. in antero-posterior extent, approximately as wide as it is long and slightly higher, dorso-ventrally, than it is wide—that is, somewhat conical in shape.

The spermatheca is a sac of tubules. The storage tubules, where the spermatozoa lie in season, are flask-shaped, from seven or eight to sixteen in number, and they converge into one central tubule which bends abruptly and opens into the cloaca. The sac is of heavily pigmented connective tissue. In the posterior wall and the floor of the sac this is thickest, most coarse and black, and the flask ends of the tubules are imbedded in the mass as in sockets. The coarse, pigmented tissue thins out in the anterior region and there is none of it in proximity to the necks of the flask-shaped tubules or the central tubule.

The extreme caudal end of the sac is but loosely connected with the cloacal wall and surrounding tissues, being held in place by scattered coarse strands of connective tissue. But approaching the mouth of the organ, where the pigmented floor thins out, it becomes more a part of the cloacal structure, the smooth musculature of the cloacal walls merging with the similar fibers of the interior of the spermatheca.

The arrangement of the tubules is noteworthy. The flask-shaped ends lie in the floor of the sac imbedded in pigmented tissue. Some are quite anterior, being anterior to the plane of the opening of the central tubule into the cloaca. But these most anterior tubules, one on each side of the median line, have their sockets of pigmented tissue though pigment is otherwise sparse in this region. The necks of the flasks converge in the posterior end of the sac and form the central tubule which runs dorsally anterior. Thus some of the flasks have very long and narrow necks. The convergence of the necks occasions a large reservoir which narrows into the central tubule proper.

The lining of the whole system of tubules is of most delicate columnar epithelium which extends into the cloaca in the region around the mouth of the central tubule. It is a markedly different tissue from the mucous lining of the cloaca. There is no mucous secretion from the spermatheca; nor at times is there any secretion of any kind as far as can be ascertained by staining reactions. Save for the spermatozoa during the breeding season the lumina may be clean and clear. Some series, however, show a faint indefinite substance, rather stringy, in the flask ends. The necks and central tubule may show slight traces of the matter also, though it is rare in the central tubule which is generally unusually clear.

There is a slight differentiation in the lining tissue in different parts of the system. In the blind ends the cells are long. The bases are densely granular with large nuclei. The apices stain faintly; they seem filled with minute, transparent globules. The apices are irregular—they do not form an even border for the lumen. They give a picket-fence appearance. In some series these inner halves of the cells are shrunken so that their ends appear as amœba-like processes, or as if they were in an exhausted state. Yet these conditions occur during the fall and winter months during which it is to be supposed that there is no reason for activity of the cells as no spermatozoa are present.

The cells of the necks of the flask tubules are shorter than those of the blind ends and produce a much more even border—that is, there is no appearance of shrinkage of the cells. The apices are rounding. It is in the central tubule, however, from the region of the convergence of the necks to the area surrounding the mouth of the cloaca that there is the most marked difference from the lining of the flask ends. The appearance of the central duct is constant at all seasons. These cells do not differ as to bases but the apices are very slightly longer and stain even more delicately than do those of the flask ends—there is no globular appearance. The apices fit perfectly together and form a smooth, even surface for the lumen. No variation of this condition has been noted in any mature spermatheca.

The size of the lumen of the central tubule varies—it is a reservoir at the convergence of the necks. In what are presumed to be very old animals there are two of these reservoirs formed by the necks of each side; these join, narrow and become the central tubule. The lumen of this tubule near the opening into the cloaca is often very small—about 1/100 mm.—but it may be as wide as 4/100 mm. The lumina of the necks vary around 1/100 mm. and those of the flask ends are usually about 6/100 mm. in diameter.

The dense coat of pigmented tissue, covering and binding in place the tubules, has been spoken of. This coat is thin, sometimes of but a few strands on the roof of the spermatheca. It is along this dorsal region that the central duct lies. This duct and the necks are surrounded by massed smooth muscle fibers which do not differ from the smooth muscle of the walls of the cloaca.

Unless injected, it is difficult in amphibian material to study the blood supply of an organ. Red blood corpuscles stain vividly with eosin and when present in numbers indicate blood vessels and capillaries even when dense pigment is present. In one series of a spermatheca which is on the verge of maturity many blood corpuscles have remained in this region. It is evident that the blood supply is rich. Corpuscles running single file everywhere throughout the region of the spermatheca indicate a close network of capillaries and some larger blood vessels are present. Only injection would show certainly, however, just where these vessels branch from the dorsal aorta which lies immediately above the spermatheca.

The mating of *Eurycea* takes place in the spring. There have been no spermatozoa found in the spermatheca during the fall and winter months. April may be taken as the average mating season though there must be as variable a season for mating as there is for egg-laying and many early and late dates have been recorded for *Eurycea* eggs.

Spermatozoa are found in the flask tubules usually in dense, orderly whorls similar to the groups found in the vas deferens of the male. They may be, however, scattered and tangled. No spermatozoa have been found in the central tubule in any series I have sectioned and in but one are there any in the necks of the tubules. This is a sagittal series and some sections, in one side of the spermatheca, pass longitudinally through a flask and its neck. The spermatozoa are streaming in a mass from the flask into the neck. But other sections show that they have not proceeded as far as the central duct.

Flask tubules containing spermatozoa show a modification of the epithelium. The tall columnar cells are unrecognizable. There is instead a narrow row of flat cells utterly different in appearance from columnar cells. The delicate part of the cells has completely disappeared and no ragged nor shrivelled edges even indicate its former presence. The width of these cells is 1/100 mm. in comparison with measurements of 3/100 and 4/100 mm. during the non-breeding periods.

Some of this difference might be accounted for by presuming that the tubules are stretched by the mass of spermatozoa contained within them. However, there does not seem to be any such stretching. Measurements of the diameter of the tubules during the breeding season are normal corresponding to the size of the spermatheca. Also when the flasks are cut squarely through, the whorl of spermatozoa is shown lying in the lumen with no adherence to the walls. There is no evidence of crowding or packing.

II.

Development.

The above description is of a typical mature spermatheca following at least one season of egg-laying. The organ in some specimens which are larger and presumably older differs in some details and will be discussed at the end of this part on the development.

The development of the spermatheca is to be considered with reference to the growth of the cloacal region also. There is a great deal of change in the whole region from the time of metamorphosis to sexual maturity.

The youngest specimen prepared was a 45 mm. female identified by Mrs. Wilder as a stage in early metamorphosis. There is no indication of any spermathecal tubules though the beginning of some cloacal glands may be apparent. In this paper the term "cloacal glands" is applied to the mucous glands of the cloaca. Kingsbury speaks of "ventral glands" but since many of the tubules are in the lateral walls of the cloaca and far dorsal, some beginning near the walls of the spermatheca, the more general term "cloacal glands" may be accurate enough. The cloaca at this stage is very simple; the walls are smooth and the only projection or fold is a small median papilla.

Other young animals prepared are 58 mm., 59 mm., 64 mm., 66 mm. and 68 mm. total length. But after metamorphosis there is no definite guide to the age of the animals as length is not a criterion and the size of the gonads is variable in all ages at various seasons, and within this range of lengths mature organs are found. A 67 mm. female killed in April was functional with spermatozoa in the flask tubules. A 68 mm. animal killed in June had a mature spermatheca which undoubtedly had been functional at least during the recent breeding season. Another April specimen, 69 mm. long, contained spermatozoa. Roughly speaking, size up to 68 or 70 mm. indicates sexual immaturity. Animals longer than 70 mm. will generally have the mature organ. Exceptions must be numerous considering the variable length of individuals at metamorphosis (I. Wilder '24).

In a 58 mm. animal identified by Mrs. Wilder as in advanced metamorphosis there are four distinct tubules in the dorsal region of the cloaca. They are in pairs—two on each side of the median line and one pair posterior to the other. These tubules are made up of a very small group of cells closely grouped and darkly stained with barely perceptible lumina and short ducts. The ducts very nearly reach the thick walls of an invagination of the cloacal wall which is the anlage of the central tubule. Several smaller but otherwise similar groups of cells appear more ventral but are most probably not part of the spermatheca group.

A 59 mm. animal in the incipient metamorphic stage also shows two pairs of tubules, one posterior to the other. Although this animal is not as far advanced in metamorphosis as the 58 mm. individual, the spermatheca anlage is in a more advanced condition. The lumina of the tubules are definitely open, the necks curve somewhat dorsally toward the median line and reach the walls of the central tubule. The opening of the central tubule into the cloaca is minute and the very short duct above is broader in diameter. Columnar epithelium is already present lining this little chamber. There is no sign of the columnar epithelium in the necks or dense cells of the two primary tubules.

A 64 mm. animal, an adult probably recently transformed, shows another pair of tubules and otherwise little change in the spermatheca development, though the cloacal glands are greater in number and length and the cloaca is beginning to form folds of the walls.

In a 66 mm. animal there is another pair of tubules thus making four tubules on a side. The tubules seem to be all of the same size; the blind ends show an open lumen; the necks are solid cords. The central tubule is no larger in size and extent that it was in the 58 and 59 mm. animals but it is further removed from the cloacal wall—more dorsal. There seems to be no opening at all into the cloaca. It is not probable that any sections are lost in this region which might account for not finding an opening. The organ at this time is functionless and is developing slowly; on the other hand the cloaca is developing steadily, that is, the walls are becoming folded and glands are enlarging. The spermatheca group of tubules is left behind until the approach of sexual maturity.

A 68 mm. animal was sectioned horizontally and badly torn by inability to fix the contents of the rectum, but there is discernible the same primitive condition as of the 66 mm. female. The number of tubules has increased; five pairs are certain but six may be present. All are in the same stage. In all these series there is no way of determining the primary pair of tubules nor the latest formed.

From the first few rudimentary tubules with their cord-like necks and small central tubule and the whole without any sign of pigment, to the pigment-walled mature spermatheca which occurs in so many 68–72 mm. females and in practically all of them of greater length, seems a great jump and implies rapid growth in a short period most probably just preceding the first egg-laying season. The author regards it as a piece of good-luck to have found, simply by chance sectioning, as there seems to be no other method of determining age at this period, a series which is intermediate between the young and the mature spermatheca. This was a 71 mm. female caught and killed in September.

In this spermatheca the average number of tubules found in a mature organ is not complete (though at least one functional organ in this study was observed with no more) and only one pair, one tubule on each side of the median line, is mature in size and cell structure. The necks of these two largest tubules are well developed: the lumina appear at first sight to be open but upon higher magnification they are seen to be so filled with the faintly stained apices of the columnar cells as to be virtually closed tubes. The remainder of the tubules and their necks are in various stages of development.

A most marked development of the central tubule is evident. This seems much out of proportion to the same duct in the fully mature organ. The extent is less, that is, the tube is short and does not extend far posterior, but the diameter is wide. Above the short, stout entrance the duct immediately widens into a very broad chamber into which the necks empty. Most of the necks being rather immature their confluence is not so graceful as is typical in older spermathecæ. Also the cells of the columnar lining of the central tubule have not the length and delicacy of apices peculiar to this duct in all later stages, and consequently they are not so conspicuous.

Lastly pigment is present in quantities but not in the arrangement of the mature organ. Sockets around the ends of the two largest tubules are being formed. Around some of the smaller tubules also it has gathered in tangled networks, but around the largest pair it is more concentrated—coarse, thick strands. Aside from these foci the pigment is scattered and the pigment producing cells still visible. The definitely bounding walls of the organ are not formed.

The spermatheca just described measures slightly less than 1/3 mm. in length and must suffice to complete the developmental series. But a word may be said about the largest spermatheca observed during this investigation. The animal was a 95 mm. female killed in September. Unfortunately for the series as a whole it was sectioned horizontally but the spermatheca is perfect. Eighteen tubules were counted and, although there is no apparent symmetry in the bilateral arrangement of the tubules, the necks from each side converge separately which causes two reservoirs and these, joining, form the large chamber of the central tubule. This narrows abruptly into the long, narrow dorsal passage which dips into the cloaca.

In this largest spermatheca as in some others which are presumed to be several breeding seasons of age, the central tubule extends quite to the posterior wall of the organ and, coincident, are the exaggerated curves of the necks of the flask tubules. All of the tubules lie in the floor of the organ but the most anterior have their blind end walls faced anteriorly. The necks then curve posteriorly and dorsally. The epithelium in this oldest series is particularly conspicuous and the whole thing is an organ of striking beauty, in size approximately 2/3 mm. long.

III.

Relation to Cloaca.

The development of the cloaca from the simple smooth-walled stage of larvæ to the complications of sexual maturity cannot well come within the scope of this paper, but a brief summary and a short description of the mature conditions should not be out of place in explaining some of the relations of the spermatheca.

The most distinctive feature of the cloaca after metamorphosis, aside from the development of the mucous glands, is the formation of a short, dorsal papilla midway between the vent and the opening of the oviducts. This lengthens antero-posteriorly becoming a fold and also deepens until it appears in sections like an icicle pendent from the roof of the cloaca. Until a period shortly preceding sexual maturity the walls of this fold are comparatively smooth. Then they become folded and especially modified at the tip. The tip broadens into a flat surface with two wings which extend close to the lateral walls of the cloaca so as to form two virtually closed dorsal passages through the cloaca up to the opening of the spermatheca where the entire fold ends. The fold undoubtedly functions as an egg guide causing a kind of continuation of the oviducts into the cloaca, but the function of the two small blind pouches in the posterior region of the fold is unknown.

Although deep folds and spurs in the walls of the cloaca admit of great expansion of the region it does not seem possible that more than one egg could pass at a time, and during the progress of this egg down one side of the cloaca corresponding to the oviduct from which it has proceeded the other passage must be entirely occluded by the pressure of the central fold against the opposite wall. Thus each egg after being squeezed through this crowded region of the cloaca arrives in the larger, freer space at the opening of the spermatheca. The distance from the opening of the oviduct to the mouth of the spermatheca is not over one half a millimeter.

The posterior wall of the fold and an extended area of the dorsal wall of the cloaca surrounding the opening of the spermatheca is lined with the delicate columnar epithelium otherwise singular to the central tubules of the spermatheca. The peculiar function of these cells can be guessed at; they secrete a substance which attracts spermatozoa. During egg-laying the smooth musculature of the spermatheca is probably affected by the convulsions of the entire region and spermatozoa are forced out from the necks of the flasks into the central tubule. Thus disturbed they may swim about and into the cloaca within the secretions of the columnar cells, however, thus preventing any loss. This secretion, as has been stated before, seems not to be mucin or any staining substance but is probably acid. Pfeffer has shown that malic acid is a common attraction for spermatozoa in ferns and Jordan ('91) believes it must be responsible for the attraction of spermatozoa to the spermatheca.

But the substances surrounding the egg as it is released into this region of the cloaca must in some manner offer greater attraction and the egg is probably surrounded with a numerous gathering of spermatozoa. It is not known whether one of these will penetrate the egg membrane immediately before it leaves the cloaca, or whether the whole group will promptly be expelled from the vent, one sperm gaining entrance and the rest perishing during attachment. There is no record of newly laid eggs being examined for impotent spermatozoa adhering to them.

The theory of the attraction of the columnar cells of the area around the mouth of the spermatheca also accounts for the spermatozoa being drawn up into the spermatheca at the time of reception instead of attempting the larger passages of the cloaca, oviducts or even rectum.

IV.

Summary.

The spermatheca of *Eurycea bislineata* is a compactly walled-in set of tubules in the arrangement of a miniature "cat-o'-nine-tails"; the tails are the bulbed or flask-like storage tubules and the long curved handle is the central tubule which is both entrance and exit for spermatozoa. The organ develops slowly in the median dorsal wall of the cloaca during and after metamorphosis until the autumn before the first egg-laying season, when, more rapidly, the majority of the tubules are formed, pigment appears forming the walls and sockets for the tubules and the columnar lining of the system is completed, proliferating back, probably, from the central tubule.

The author expresses profound appreciation for the help and advice of Dr. Harris Hawthorne Wilder and Mrs. Inez Whipple Wilder in the preparation of this paper.

Material and Technique.

Aside from dissections of fresh and preserved material, fifteen female *Eurycea* were sectioned. In size these vary from 45 mm. to 95 mm. total length. Specimens were killed in September, October, November, February, April and June. All were decalcified and sectioned entire—from the ovary to a point caudal to the vent of the cloaca—to preclude any distortion of the cloacal region. Bouin's fixative was generally used though some of the young animals were preserved in formalin. Fourteen were stained with hematoxylin-eosin and one with muci-carmine. Most of the series are transverse; two are frontal and two sagittal. The drawings are made with a projection apparatus.

ABBREVIATIONS USED.

ant	anterior	*nc*	nerve cord
ap	anterior pouch	*nt*	notochord
bl	bladder	*ov*	oviduct
cg	cloacal glands	*post*	posterior
cl	cloaca	*pu*	pubis
ct	central tubule	*pw*	posterior wall of spermatheca
dor	dorsal	*rc*	rectum
f	dorsal fold of cloaca	*sp*	spermatheca
ft	flask tubule	*spz*	spermatozoa
ms	mesonephros	*ur*	ureter
n	neck	*vent*	ventral

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EXPLANATION OF FIGURES.

Plate I.

Fig. 1. (Mag. × c. 85.) Fig. 1 is a diagrammatic drawing of a young functional spermatheca with part of the pigment sac removed. The diagram is based upon the study of the spermatheca of the 69 mm. female of Fig. 3 and the immature spermatheca of a 71 mm. specimen described in the text. An average of eight tubules would be typical of this stage. The necks bend but slightly posterior and some are anterior in direction. At their convergence the central tubule is extremely large but short, antero-posteriorly, and narrows as it dips into the cloaca. The posterior wall and floor of the spermatheca are densely pigmented and sockets enclose the bulb of each flask tubule.

Fig. 2. (Mag. × c. 85.) Fig. 2 is a diagrammatic drawing of an older spermatheca. The sac wall is removed and half of the tubules are shown, bisected to show their contents—the coiled spermatozoa in the flask tubules. These tubules lying in the floor of the sac in pigmented sockets bend posteriorly and dorsally to converge into the central tubule. The reservoir formed by this convergence narrows and the duct passes anteriorly through the dorsal part of the sac where it bends to pass into the cloacal wall. The diagram shows this portion of the central tubule anterior to all the flask tubules but in many cases it passes between the two most anterior flask ends. See Fig. 7. (Mag. × c. 85.)

Fig. 3. (Mag. × 19.) Fig. 3 is a para-sagittal section through the region of the rectum and cloaca of a 69 mm. female to show the orientation of the spermatheca. The plane of sectioning is not quite true so that the anterior region of this drawing is very nearly median-sagittal showing the nerve cord, notochord, rectum and bladder. The posterior half of the drawing shows the end of the mesonephros, one oviduct, the spermatheca and the cloaca almost obscured by the many folds of its walls. It is to be noted in this figure that the mesonephros does not extend as far posterior as the spermatheca. This may be compared with transverse sections shown in other figures. In some individuals the mesonephros lies dorsal to the spermatheca and in others it ends more anteriorly. It is not certain whether these variations are due to changes or varying lengths in the mesonephros itself or if they might be due to the influence of the spermatheca.

An "*f*" placed below a fold separating the oviduct from the lower part of the cloaca is part of the fold represented in Fig. 10, transverse section.

The spermatheca in this specimen is young, of the type represented in Fig. 1. It is higher, dorso-ventrally, than it is long, antero-posteriorly. There are few tubules and the necks of these do not bend posteriorly. Through a median section the central tubule is large as in Fig. 1, but this section is through one side of it. The flask ends are filled with spermatozoa and in this section is the tubule described in the text from which the spermatozoa are streaming into the neck. The lining epithelium of these tubules is flat, unlike the delicate columnar cells pictured in the one flask tubule of Fig. 9, or in Fig. 5.

"*ap*" on this figure indicates an interesting fact about the mature cloaca that is very puzzling in transverse sections through the region—an anterior pouch, although this section is only through the wall of it. The anterior pouch is a ventral region of the cloaca separated from the anterior dorsal region by a band of muscle between the bladder and the cloaca. The region is left blank in the figure and indicated by "*m*." Ventral dissections of the cloaca did not show this particular and in transverse sections it appeared so startling that its function was not guessed.

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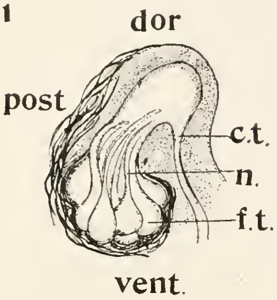
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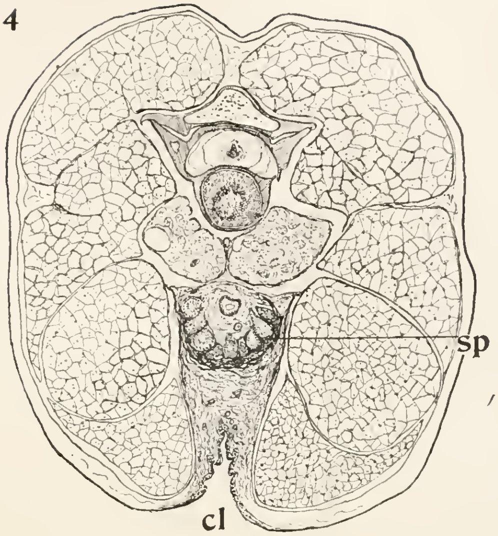

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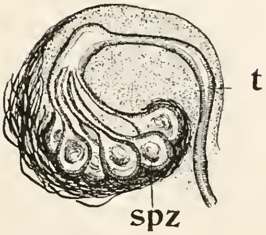

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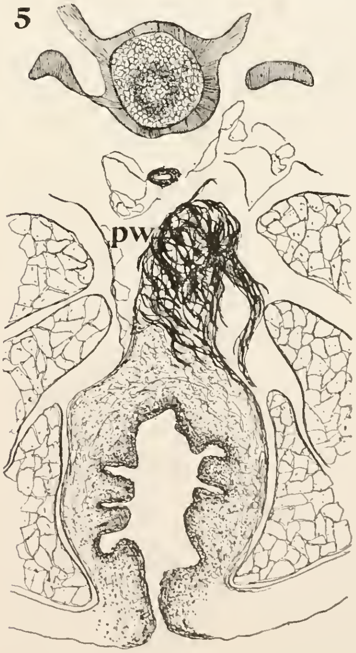

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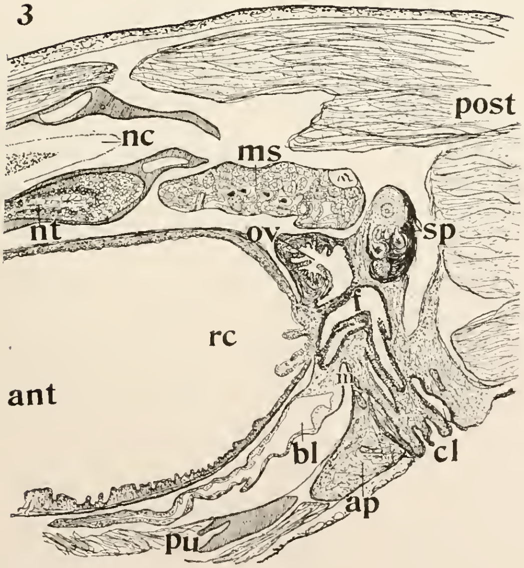
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VERA KOEHRING.

From a sagittal view, however, it is very simply explained. During egg-laying the rectum and bladder are pushed anteriorly, the folds of the cloaca smooth out and the anterior pouch is drawn down as the vent is stretched open. It is but another rather larger fold of the cloaca.

FIG. 4. (Mag. × 17½.) Fig. 4 is a transverse section through a 68 mm. female midway through the spermatheca. The mesonephros lies above the spermatheca. In the floor of the spermatheca lie the flask tubules. A few necks are evident. The central tubule is near the dorsal wall. This is an animal killed in June; the tubules are empty and the columnar cells long. Although this animal is short in body length the spermatheca and cloaca show the maturity of several seasons of breeding.

PLATE II.

FIG. 5 (mag. × 40), FIG. 6 (mag. × 41½), FIG. 7 (mag. × 42). Figs. 5, 6 and 7 are regions of the spermatheca of a 70 mm. female. This organ has not as many tubules as that of the 68 mm. spermatheca of Fig. 4 nor is the cloaca as complex. On the other hand, there are more tubules and more pigment than in the 69 mm. spermatheca of Fig. 3. The significance of these variations after maturity is not known.

Fig. 5 is a transverse section through the posterior wall of the spermatheca. Fig. 6 is through the region where the necks are converging into the central tubule. Fig. 7 is through the anterior part of the organ; the central tubule bends to open into the cloacal wall and the most anterior flask tubules lie on either side.

FIG. 8. (Mag. × 50.) Fig. 8 is a transverse section through the anlage of the spermatheca in a 59 mm. female. One pair of flask tubules is shown and the central tubule in which the columnar epithelium has developed. The short, cord-like necks do not show in this section.

FIG. 9. (Mag. × 86.) Fig. 9 is a frontal section through a 95 mm. female. The necks converge into two groups which join to form the central tubule. One flask tubule is shown with the characteristic ragged-looking but nevertheless very delicate epithelium. The necks are similar to the epithelium of the central tubule. The oviducts and one ureter are in the anterior part of the drawing.

FIG. 10. (Mag. × 26½.) Fig. 10 is the region through the cloaca of the 68 mm. individual of Fig. 4 several sections anterior to the opening of the spermatheca. The central fold pendent from the wall of the cloaca divides the dorsal region, and serves as an egg guide.

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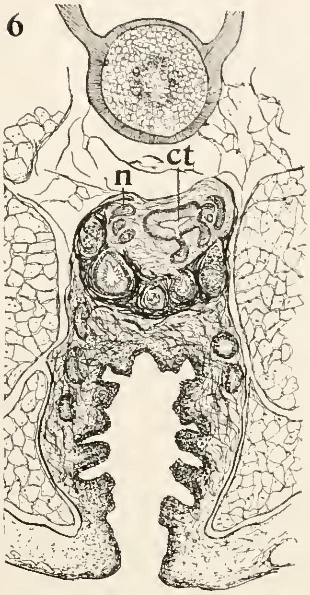

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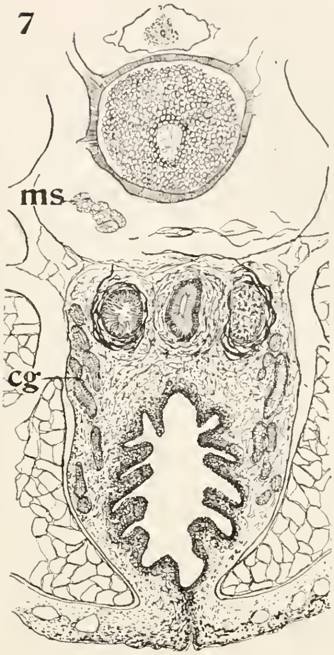

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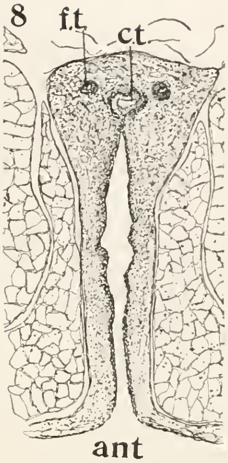

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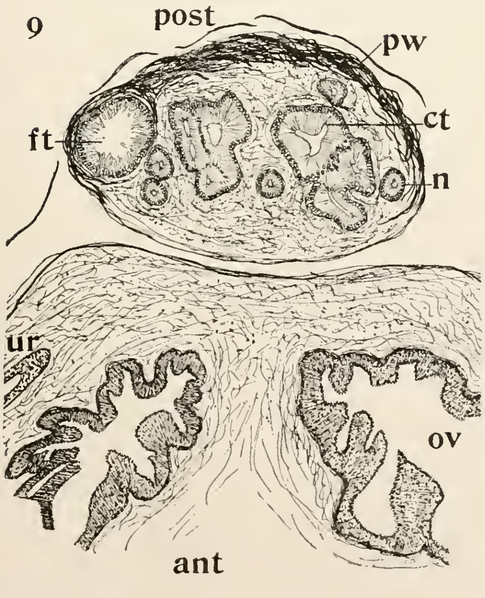
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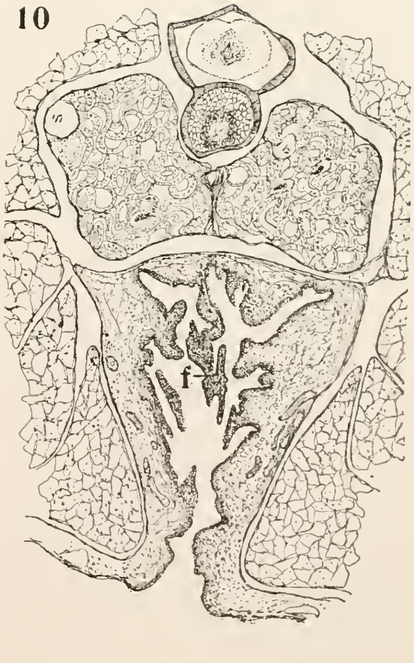

THE INHERITANCE OF A MACULA MUTATION CONCERNED WITH ELYTRAL SPOTTING AND LATENT TRAITS IN THE MALE OF *BRUCHUS*.[1]

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A conspicuous, bilateral, three-character pattern is found on each elytron of the normal wild type female. It consists of two circular black spots placed one anterior and the other posterior, and a thin horizontal line of white pubescence along the inner edge. The elytron of the wild male is unmarked, the four black spots and the two white lines being absent. This condition enables the sexes to be easily distinguished and makes the wild *Bruchus quadrimaculatus*, Fabr., sex-limited.

In this culture, on October 3, 1922, at the University of Oklahoma, there appeared a male insect bearing indistinct black spots and white pubescence, a pattern similar to that of the female described above although less perceptible. This mutation was designated "macula." Approximately a year of inbreeding and selection was required before homozygous macula cultures could be assuredly separated from the wild type in which they originated, because the macula female and the wild female are identical in both homozygous and heterozygous cultures. The difference between the unmarked wild type male and the spotted, macula male was therefore the only visible criterion available for these selection tests.

The macula mutation is represented homozygously by the genes, MM, and its recessive allelomorph, spotted females and non-spotted males of the normal insect, homozygously by the factors, mm.

In the first test a (MM) female, homozygous for spotted males and females was crossed with a normal (mm) male, homozygous for non-spotted males and spotted females. The offspring from

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[1] It is a pleasure to acknowledge my indebtedness to Dr. Frank R. Lillie, Director, for the privilege of utilizing the many excellent facilities of the Marine Biological Laboratory.

22 separate pairs, totaled 422 heterozygous, spotted (Mm) females and 412 heterozygous, spotted (Mm) males. This proves that the macula trait, spotted males and females, is dominant to the normal character, non-spotted males and spotted females. Complete data for this test is tabulated in Table I.

Table I.

P1: 1 MM female, homozygous for spotted males and females × 1 mm male, homozygous for non-spotted males and spotted females.

F1: Offspring:

Pair No.	Females Spotted.	Males Spotted.	Pair No.	Females Spotted.	Males Spotted.
	(Mm)	(Mm)		(Mm)	(Mm)
3	15	16	98	15	15
2	20	29	99	32	42
17	28	14	104	19	9
19	33	19	110	1	1
36	5	17	116	6	5
44	38	32	5	13	10
51	11	13	9	0	3
54	24	27	10	10	0
59	24	38	106	19	18
60	25	16	112	27	35
94	18	14	120	34	39
			Totals	422	412

Table II.

Reciprocal Cross.

P1: 1 mm female, homozygous for non-spotted males and spotted females × 1 MM male, homozygous for spotted males and females.

F1: Offspring:

Pair No.	Females Spotted.	Males Spotted.	Pair No.	Females Spotted.	Males Spotted.
	(Mm)	(Mm)		(Mm)	(Mm)
1	11	8	79	31	34
10	1	2	80	37	29
34	15	30	81	33	16
39	11	11	88	18	16
47	24	19	90	24	23
48	3	6	92	51	56
49	15	9	109	19	27
63	28	46	123	22	25
64	6	7	6	2	0
68	7	6	9	9	5
69	15	16	7	49	27
78	44	46	8	12	9
			Totals	488	483

TABLE III.

F$_1$: Mm heterozygous spotted female × Mm heterozygous spotted male.
F$_2$: Progeny:

Pair No.	Females Spotted.	Males.	
		Spotted.	Non-spotted.
	(MM, 2 Mm, mm)	(MM, 2 Mm)	(mm)
4	23	19	9
87	37	30	7
126	15	10	3
127	45	33	15
128	30	19	4
11	14	16	4
3.1	26	15	6
3.4	7	6	1
3.7	48	31	13
19.1	9	11	3
19.9	34	20	5
44.3	36	27	8
54.15	22	16	3
54.31	32	25	7
71.5	4	5	2
87.3	63	44	15
99.5	16	13	3
104.3	17	18	5
116.1	8	4	1
116.3	17	12	3
2.6	21	17	8
2.7	9	7	2
54.20	33	29	9
59.6	54	37	14
60.6	13	11	4
5	49	39	16
20	37	22	9
62	7	5	2
63.1	75	58	20
69.1	21	17	2
69.5	20	15	5
81.5	26	12	3
91.2	43	39	19
108.4	55	45	16
109.3	27	28	8
109.4	69	59	22
123.2	34	35	10
123.3	31	20	5
123.7	79	68	16
7.2	21	17	4
7.3	24	20	6
7.4	7	8	2
6.2	19	12	4
Totals	1,079	994	323

The second experiment was the reciprocal of the previous one. A (mm) female, homozygous for spotted females and non-spotted

TABLE IV.

BACK CROSS TEST

P_1: Mm heterozygous spotted female × mm homozygous non-spotted male.
F_1: Progeny:

Pair No.	Females Spotted.	Males.	
		Spotted.	Non-spotted.
	(Mm, mm)	(Mm)	(mm)
4	23	13	10
71	31	19	20
86	32	16	17
96	10	4	3
105	0	1	2
119	22	7	7
125	21	17	16
3.1	26	13	10
3.2	32	9	10
3.5	10	7	6
3.6	30	16	10
17.3	12	7	4
198	18	11	15
51.4	3	2	3
51.6	36	18	13
54.1	20	22	23
54.2	45	20	12
54.4	25	14	13
54.14	7	4	3
54.16	37	18	18
54.30	28	10	9
60.1	131	47	69
60.3	31	11	19
60.4	27	14	14
60.8	43	22	23
60.9	30	13	19
85.3	34	20	16
86.2	74	40	52
87.1	126	68	50
94.4	24	7	11
94.5	13	4	6
98.2	31	5	6
98.3	29	10	12
99.1	23	17	10
99.2	103	34	34
99.3	4	3	3
99.4	96	44	42
119.1	11	7	9
125.3	37	18	11
127.1	52	24	24
128.4	11	3	4
Totals	1,375	679	658

males was bred to a (MM) male, homozygous for spotted males and females. The F_1 offspring from 24 separate pairs gave 488 heterozygous, spotted (Mm) females and 483 heterozygous, spotted (Mm) males. This test further shows the dominance of the spotted trait to the normal. Furthermore this experiment, when compared with the previous one, proves that the character is not sex-linked. Table II. gives the data.

The third test concerned heterozygous, spotted (Mm) females and heterozygous, spotted (Mm) males. The F_2 progeny from 43 F_1 single pairs totaled 1,079 spotted (MM, Mm, mM, mm) females, 994 spotted (MM, Mm, mM) males, and 323 non-spotted (mm) males. The result approaches a 4 : 3 : 1 sex-limited ratio, which shows that the spotted females appear identical, and that macula is dominant to normal. This data is tabulated in Table III.

The fourth experiment is a back cross with the F_1 hybrids, (Mm), heterozygous insects, and the normal type (mm) weevils. The parents were heterozygous, spotted (Mm) females and homozygous, non-spotted (mm) males. From 41 single pair matings 1,375 spotted (Mm, mm) females, 697 heterozygous, spotted (Mm) males, and 658 homozygous, non-spotted (mm) males, were obtained. The ratio, therefore, approximates a sex-limited 2 : 1 : 1. This result indicates further that normal spotting is recessive to macula. The data is listed in Table IV.

The fifth experiment was another back-cross test, the reciprocal of the previous one. Homozygous, normal, spotted (mm) females were mated with heterozygous spotted (Mm) males. From the 43 different pairs a total of 1,415 spotted (Mm, mm) females, 786 heterozygous spotted (Mm) males and 798 homozygous non-spotted (mm) males were obtained. This gave a 2 : 1 : 1 sex-limited ratio, or actually a 1 : 1 ratio, since the females appeared alike. Spotting is dominant to the normal type. Complete information is found in Table V.

In the sixth test heterozygous spotted (Mm) females and homozygous spotted (MM) males were used. The offspring from 26 separate pairs totaled 701 spotted (MM, Mm) females and 654 spotted (MM, Mm) males. This demonstrates the dominance of the macula character to its recessive normal trait, spotted females and non-spotted males. This data is compiled in Table 6.

TABLE V.

BACK CROSS TEST: RECIPROCAL CROSS.

P_1: mm homozygous wild type female × Mm heterozygous spotted male.
F_1: Offspring:

Pair No.	Females Spotted.	Males.	
		Spotted.	Non-spotted.
	(Mm, mm)	(Mm)	(mm)
6	33	30	27
6.1	2	4	4
31	27	20	15
91	33	18	19
108	20	8	9
100	24	10	13
103	41	24	19
1.2	34	14	15
20.7	16	8	9
31.6	31	19	26
34.3	16	9	6
34.2	11	3	4
39.1	25	16	19
78.1	31	33	38
78.3	17	4	4
79.1	29	11	17
79.3	48	19	19
79.7	13	6	4
81.4	33	15	18
88.2	62	33	33
88.7	15	7	11
91.1	31	16	14
91.3	95	37	34
91.6	34	60	51
92.1	15	7	12
92.5	73	22	28
92.6	157	83	78
108.1	5	7	12
123.1	67	28	29
123.4	40	19	16
64.1	6	7	5
64.2	8	10	11
112.1	33	13	16
112.2	64	37	31
9.10	42	19	23
54.11	3	2	3
54.28	30	13	17
82.2	25	15	11
116.5	29	12	10
128.1	27	23	17
10.10	8	4	5
101.2	15	10	10
Totals	1,415	786	798

The seventh experiment was the reciprocal of the previous one. The parents were homozygous, spotted (MM) females and

Table VI.

Back Cross Test.

P1: Heterozygous, Mm, spotted female × homozygous, MM, spotted male.
F1: Offspring:

Pair No.	Female Spotted.	Male Spotted.	Pair No.	Female Spotted.	Male Spotted.
	(MM, Mm)	(MM, Mm)		(MM, Mm)	(MM, Mm)
20.3......	1	1	47.5.......	19	17
48.2......	4	5	49.1.......	21	24
62.4......	17	15	49.2.......	3	4
69.4......	9	4	47.7.......	5	9
78.6......	70	56	47.8.......	30	34
80.5......	3	3	48.1.......	11	6
81.1......	33	16	68.5.......	31	32
88.5......	3	1	68.6.......	28	40
88.6......	22	5	68.7.......	29	25
92.7......	131	149	79.5.......	37	32
47.9......	37	25	80.2.......	21	30
47.3......	43	34	80.4.......	36	49
47.4......	28	20	81.8.......	29	18
			Totals....	701	654

heterozygous spotted (Mm) males. The offspring from 15 pairs, gave 236 spotted (MM, Mm) females and 213 spotted (MM, Mm) males. Hence, both sexes were homozygous for the macula (MM) genes, and heterozygous for the macula (M) gene and normal (m) factor. The macula mutation is dominant to the wild type. Complete data is presented in Table VII.

Table VII.

Back Cross Test.

Reciprocal Cross.

P1: Homozygous, MM, spotted female × heterozygous, Mm, spotted male.
F1: Offspring:

Pair No.	Female Spotted.	Male Spotted.	Pair No.	Female Spotted.	Male Spotted.
	(MM, Mm)	(MM, Mm)		(MM, Mm)	(MM, Mm)
17.1	28	23	94.6	10	5
17.2	4	1	51.8	6	9
51.2	21	10	54.12......	3	0
51.3	2	2	54.21......	36	33
51.11.....	18	18	126.4	9	17
59.2	26	18	126.8	18	18
59.3	23	27	101.	29	31
71.4	3	1			
			Totals.....	236	213

The above series of experiments prove that the two elytra traits, normal and macula, differ in the male phenotypes only, since the factors for the macula (MM) mutation and the wild (mm) type appear identical for both female traits. These females are alike phenotypically but deviate genotypically, since the normal female has the same black four spotted pattern as its mutant, the macula female. Again, normal is recessive to macula.

Many sex-differences are detected in insects, occurring most frequently in Diptera; *Drosophila* probably furnishing the greatest number, with Lepidotera next, then Coleoptera. This sequence appears, as the result of the amount of genetic study directed upon these species. Many of the sex-limited traits in *Drosphila* are less distinct in one sex than in the other, thus differing somewhat from *Bruchus*, in which there is no visible manifestation of such characters in the male, except for the macula mutation.

Examples of these sex-limited traits for *Bruchus*, in which the male elytra is a non-spotted tan, have been previously demonstrated, hence they will be merely mentioned in this relation. The first mutations discovered (Breitenbecher, 1921) consisted of red, black, white, and tan elytra colors apparent only in the females. The respective male for each of these four female cultures was a non-spotted tan, the wild type elytra color. The four traits are multiple allelomorphs. A second non-visible trait in the male occurs with mosaic females (Breitenbecher, 1922). These females have elytra of different colors, often combined with varied spotting. In a third instance the males remained non-spotted tan, although the females displayed four red spots on the elytra (Breitenbecher, 1923). This character was dominant to the normal. Another mutation, in which the male showed a complete absence of the trait, was that in which all females were apterous. The males were fully winged. This was a recessive character (Breitenbecher, 1925). Lastly, another mutation, never visible in the male, was called "piebald." Here bilateral asymmetry was manifested since the females were of two types, about equal in numbers. One group had two red spots on the left elytron and two black spots on the right; the other group had two black spots on the left elytron and two red ones on the right. When the two types of females were added, the trait was found to be inherited as a recessive to normal (Breitenbecher, 1925). Every male, for the entire list of characters enumerated, had non-spotted tan elytra similar to the wild type.

It is improbable that such latent characters in the male are caused by developmental differences, because in sex-limited, as well as other Bruchid mutations, the male emerges a fraction of a day before the female. Inhibitors associated with the male complex may produce this peculiarity. Or, since the female has two X-chromosomes, and the male only one X-chromosome, the sex-limited traits may be caused by pattern, or normal, genes within the X-chromosome. These may be associated with factors in other chromosomes in a degree enabling the character to be completely manifested in the female. The one sex-chromosome of the male may not be sufficient for complete manifestation of the trait. This conception is similar to that of Bridges (1922) for *Drosophila*.

The macula and normal patterns of *Bruchus*, illustrating sex differences, may be considered as the result of identical phenotypes for the female, since the females for each trait have duplicate spotting patterns on the elytra. The mm genes in the wild male are non-visible, while the MM factors for the normal four black spots are visible in the macula male.

It is to be hoped that some mutation may occur, in which the gene concerned with the entire pattern or non-pattern trait may appear. This might assure a definite solution for these sex-limited differences in *Bruchus*.

CONCLUSIONS.

1. The macula mutation is dominant to the wild type.
2. Genes, mm, represent the wild type, having spotted females and non-spotted males.
3. Factors, MM, represent the macula mutation, in which both males and females are spotted.
4. The macula character is not sex-linked.
5. Inhibitors probably produce latent traits in the male.

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THE EFFECT OF THYROID FEEDING ON THE MOULTING PROCESS AND FEATHER STRUCTURE OF THE DOMESTIC FOWL.

HARRY BEAL TORREY AND BENJAMIN HORNING.

1.

In the course of investigations on some of the endocrines and their functional correlations in the domestic fowl, it was observed that the addition of desiccated thyroid to the ration of Brown Leghorn and White Leghorn chicks led to striking modifications in the form of the first set of rectrices, and in the course of the first moult (Horning and Torrey, 1923). The form of the rectrices was modified conspicuously by the shortening of the vane and the lengthening of the naked region of the shaft. A characteristically "quilled" feather was the result, though somewhat reduced in total length. The course of the moult was modified by the maintenance of base-to-tip continuity between first and second rectrices, in sharp contrast to the normal independent and discontinuous development of these feathers.

The results of this experimentally produced hyperthyroidism, and others to be considered in another place, were then examined for the light they might throw upon the rôle of the thyroid hormone in feather differentiation, possibly in processes of differentiation in general. Feathers afford certain advantages as material, for they give permanent records of the progressive changes that occur in the feather germ during their development. But it is only of such a period that they do give reliable records, obviously enough, for once grown, they are removed from the influence of experimental procedures to which they might earlier have been susceptible.

2.

The following experiment is typical and with the accompanying tabulation will suffice to establish the fact that desiccated thyroid given in appropriate doses produces "quilling" of the rectrices of chicks of both sexes, with or without gonads.

Fifty-two Brown Leghorn chicks, hatched March 27, were divided into four groups as indicated in Table I.: two control groups (one with and one without gonads); two experimental groups (one with and one without gonads). A week later, on April 3, the rectrices of all were pushing out. On that date daily doses of 16 mgm. of desiccated thyroid (Armour's, containing 0.2 per cent. iodine) in capsule by mouth, were begun for one group of normal chicks (Nos. 36–50). On April 17, the dosage was increased to 32 mgm. and given to the second experimental group (Nos. 51–63) also.

TABLE I.

Controls.			Thyroid-fed Chicks.		
No. of Chick.	Sex.	Rectrices.	No. of Chick.	Sex.	Rectrices.
25.......	Female	None quilled	37......	Male	2 pairs quilled
26.......	Female	" "	38......	Male	4 " "
27.......	Male	" "	39......	Female	6 " "
28.......	Female	" "	40......	Female	4 " "
29.......	Female	" "	41......	Female	2 " "
30.......	Male	" "	42......	Female	5 " "
31.......	Male	" "	44......	?	4 " "
32.......	Male	" "	45......	Male	4 " "
33.......	Male	" "	46......	Female	4 " "
34.......	Female	" "	47......	Male	3 " "
35.......	Male	" "	48......	Male	3 " "
36.......	Female	" "	49......	Male	2 " "
			50......	Fmeale	5 " "

Gonadless Controls.			Gonadless Thyroid-fed Chicks.		
No. of Chick.	Sex.	Rectrices.	No. of Chick.	Sex.	Rectrices.
64.......	Male	None quilled	51......	Male	3 pairs quilled
65.......	Female	" "	52......	Male	4 " "
66.......	Female	" "	53......	Female	? ? ?
68.......	Female	" "	54......	Male	6 pairs quilled
69.......	Male	" "	55......	Male	1 " "
70.......	Male	" "	56......	Female	2 " "
71.......	Male	" "	57......	Male	? ? ?
72.......	Female	" "	58......	Female	? ? ?
73.......	Female	" "	59......	Male	? ? ?
74.......	Female	" "	60......	Male	6 pairs quilled
75.......	Male	" "	61......	Female	1 " "
76.......	Female	? ?	62......	Female	3 " "
77.......	Male	None quilled	63......	Female	1 " "
78.......	Female	" "			

The condition of the rectrices in all groups on May 18, 52 days after hatching, is shown in Table I.

The shortening of the vane characteristic of the "quilled" feathers was due to a failure of a considerable number of barbs to differentiate proximally. There was no sign of a loss of barbs previously formed and broken off. The vane of each "quilled" feather, though abnormally proportioned as already indicated, ended below in an aftershaft of barbules surrounding the superior umbilicus, with no sign that there had ever been barbs proximal to this point.

As is well known, the barbs of a feather arise from the so-called intermediate cells of the feather germ epidermis, these intermediate cells differentiating in the growing zone into parallel ridges, which, as the feather germ elongates, run a longitudinally oblique course about the dermal core. These ridges differentiate beginning with their distal ends into the barbs and barbules of the definitive feather and are continually renewed from below.

Now the effect of thyroid feeding was to abbreviate the period of ridge formation in the growing zone, and as a consequence to suppress prematurely in that region the processes of differentiation on which barb formation normally depends, without suppressing, however, the processes on which the lengthening of the calamus depends. It is true that the total length of each of the "quilled" feathers thus produced was somewhat less than normal, but that this was not to be attributed to a direct inhibitory effect of the thyroid upon the growth of the feather germ, but rather to a precocious development of the second rectrices, will appear, we believe, from facts to be given below. It may be said here, however, that while the suppression of barb ridge differentiation occurred prematurely after thyroid, in rare cases these ridges might appear again prematurely, after thyroid, about the inferior umbilicus of the first rectrix, foreshadowing the base-to-tip connection of first and second rectrices, to be described in the next section.

3.

In size, shape and coloration—details of which do not here concern us—the first rectrices, belonging to the first few weeks of the chick's life after hatching, differ distinctly from the second, which are adult in type.

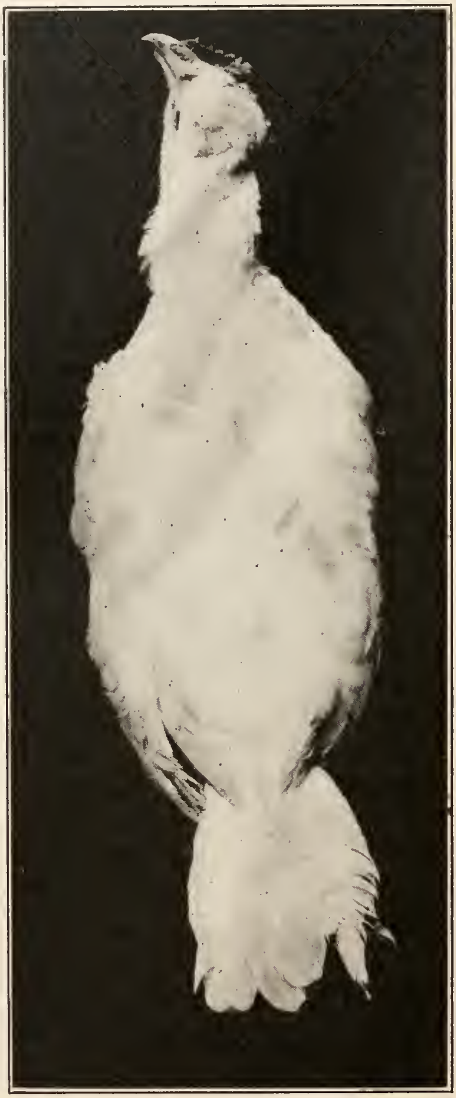

FIG. 1. White Leghorn male about 8 weeks old, with normal rectrices On the extreme right is a rectrix of the first set; all the others are of the second set.

Normally the first or infant rectrices may drop away without urging from below; or they may be pushed out by the seconds that are developing from the same follicles. In neither case, however, are the two continuous. Though the succeeding feather may arise from the same germinal papilla as its predecessor, a period of inactivity of the papilla follows the withdrawal from the calamus of the first and the rounding off of the inferior umbilicus. A new feather is then begun, within a sheath of its own.

The rounding off of the tip of the calamus of the first feather and the establishment of a discontinuity between the first feather and its successor involve certain processes of differentiation before the feather follicle and papilla become quiescent. One of the effects of thyroid feeding in our experiments was to suppress these terminal processes of differentiation by maintaining the papilla in continuous, even though, it may be, reduced activity. This continuity of process was revealed in continuity of structure —and, incidentally, settled, in this case, the question that has sometimes been raised whether successive feathers from the same follicle spring from the same papilla. Here the second obviously did spring from the same papilla as the first. If the feathers of succeeding moults arise similarly, the series, in place of a single feather, would appear to be due to a rhythmic variation in the velocity of critical reactions conditioning the development of the papilla. This variation, normally extreme and leading to discontinuity, is essentially independent of the normal thyroid secretion. The addition of thyroid to the ration under the conditions of our experiments lessened the amplitude of the rhythmic variation without determining the rhythm itself.

The "quilled" first rectrices were originally noticed in our thyroid-fed chicks as distal appendages to the members of the second set, since they were not being shed in normal fashion. Examination showed that the structure of the inferior umbilicus was unusual, the proximal end of the calamus being wide open and the plane of the orifice running ventrodorsally at a sharp angle with the axis of the calamus. Dorsally the lip of this oblique orifice was extended proximally as though the quill had been cut to form a long pen point.[1]

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[1] In exceptional cases, as in a wing quill before us, this proximal extension is not a prolongation of the median dorsal line of the calamus, but of a region perhaps twenty degrees away from it. Though the median dorsal lines of the two feathers do not lie in the same plane, there is no obvious structural twist to account for the fact.

At the tip this penlike extension was continuous with the rhachis of the proximal feather. Elsewhere the lips of the orifice, including the edges of this dorsal extension, were continuous with the distal ends of about fifteen pairs of barbs of the proximal feather. The original orientation of the barbs, which had differentiated from long spiral ridges in the wall of the cylindrical feather germ, was thus preserved, and the orifice correspondingly obscured.

The falling of the distal feather was accomplished by rupture of these connections. As a result, the distal end of the second rectrix presented a frayed appearance. This came to be recognized as a characteristic of the second rectrices of thyroid-fed birds, in sharp contrast with the smooth rounded ends of the vanes of normal birds.

4.

What had been said regarding the effect of thyroid feeding on the development of the first rectrices and the first moult applies also to wing quills, though much less frequently and with slight unessential differences in detail; such, for instance, as the obliquity of the umbilical orifice. Contour feathers are not affected, according to our observations.

The facts may be briefly summarized. Thyroid feeding inhibits: (1) the differentiation of proximal barbs and barbules, thus shortening the vane without proportionately shortening the calamus; the proximal region of the vane passing into the after shaft normally without traumatic defect; (2) the usual differentiation of the inferior umbilicus; (3) the withdrawal of the feather pulp from the calamus, and the normal inactive period of the pulp. Continuity between first and second rectrices is the result.

Thyroid feeding also (4) accelerates the differentiation and eruption of the second rectrices. This acceleration was observed first in Rhode Island Red males (Torrey and Horning, 1922). That it occurs in Brown Leghorns is shown by the facts in the next paragraph. The precocious development of the second rectrices correspondingly limits the maximum growth period of the first and probably accounts, at least in part, for the reduced length of the latter after thyroid.

Thirty-two Brown Leghorn chicks hatched April 9 were divided into two lots, each containing 8 males and 8 females. One lot was fed desiccated thyroid, beginning April 14: 10 mgm. daily for 5 days, then 15 mgm. daily. On May 22, 43 days after hatching, the moult had proceeded as shown by the following table.

Table II.

Controls.				Thyroid-fed Chicks.			
No. of Chick.	Sex.	First Rect.	Second Rect.	No. of Chick.	Sex.	First Rect.	Second Rect.
1....	Female	4 pairs	3 pairs	17......	Female	1 + 3 pairs	4 pairs
2....	Female	5 "	1 "	18......	Female	3 + 4 "	4 "
3....	Female	7 "		19......	Female	3 "	3 "
4....	Female	7 "		20......	Female	1 + 2 "	4 "
5....	Female	5 "	2 "	21......	Female	1 + 4 "	4 "
6....	Female	7 "		22......	Female	3 "	4 "
7....	Female	7 "	1 "	23......	Female	1 + 3 "	4 "
8....	Female	5 "	2 "	24......	Female	4 "	3 "
9....	Male	7 "		25......	Male	6 "	1 "
10....	Male	7 "		26......	Male	3 + 4 "	3 "
11....	Male	7 "	1 "	27......	Male	5 "	2 "
12....	Male	6 "		28......	Male	5 "	2 "
13....	Male	6 "		29......	Male	2 "	4 "
14....	Male	0 "		30......	Male	4 "	3 "
15....	Male	7 "		31......	Male	died	
16....	Male	7 "		32......	Male	3 pairs	4 "

The second rectrices recorded for the controls were just appearing. Those recorded for the thyroid-fed chicks were an inch or more longer. In chicks numbered 17, 20, 21 and 23 the variation in the notation indicates that one pair of first rectrices was "quilled" and continuous with the second; in number 26, 3 pairs were "quilled" and similarly continuous with the second.

5.

In certain respects the modifications in structure and moulting that we have described recall defects of structure and moult that have been associated with fault-barring through the work of Strong (1902), Riddle (1907, 1908, 1908*a*) and Whitman (1919).

Strong (1902) called attention to abnormal transverse bands in both remiges and rectrices of a hybrid pigeon, referable clearly to defective barbules. He also described and figured one of the body coverts, for the most part downy and carrying a

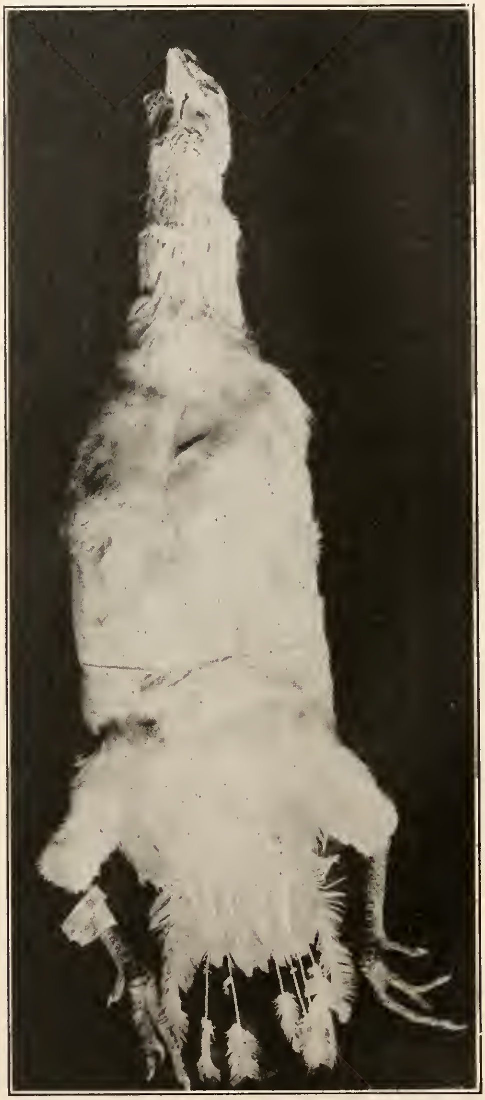

FIG. 2. White Leghorn male about 7 weeks old, that was fed thyroid from the age of two weeks. The outer pair of infant rectrices are not "quilled" and may be compared with their "quilled" mates intervening.

tassel-like distal appendage with a horny cylindrical base continuous with rhachis and barbs of the main body of the feather. The horn cylinder he interpreted as a region in which, during the development of the feather, differentiation but not cornification had been omitted.

Riddle (1907) distinguished five types of defect in feather structure, two being represented by Strong's cases, a third by a very inconspicuous transverse depression or line without defects in barbules, a fourth by constrictions in the feather germ, and a fifth by a single instance of a feather with barbs broken off at even distances from the shaft, the defect extending parallel to the rhachis.

The first three of these are of widespread occurrence among birds. Riddle discussed them in connection with what Whitman had called fundamental bars (alternating lighter and darker transverse bands of pigment) so beautifully figured in his posthumous paper (1919, pls. 72–75). He concluded (1908) on the basis of experiments, that both are referable to variations in the nutrition of the feather germ during its development, the fundamental bars being produced by diurnal changes in blood supply, the fault bars by defective nutrition through this and other agencies. In his experiments, reducing the food, feeding Sudan III, mechanical destruction of tissues and blood vessels and the administration of amyl nitrite were all effective.

We have no satisfactory evidence of variation in the blood pressure of our birds. But we have seen abnormalities in feather structure belonging to Riddle's fifth type as a direct consequence of starving two White Leghorn males. One of our adult male Brown Leghorns on a daily ration of 1 gm. of dessicated thyroid replaced three pairs of remiges with feathers of normal length but abnormal in structure and color. The vanes were narrower and proximally misshapen as though fashioned under cramped conditions. The normal color was almost entirely lacking, the feathers being prevailingly white. There appears to be no reason to doubt that directly or indirectly such defects are referable to disturbed nutrition. We have also two rectrices from an adult Rhode Island Red female nearly two years old that resembles Strong's feather with a "tassel-like appendage." One of these rectrices is marked with great regularity by transverse depressions belonging to Riddle's third type of feather defect. At the distal end of the "appendage" two or three of these are definitive fault bars, being represented by actual defects in barbule formation. Toward the base of this "appendage," as the vane narrows, they become more and more closely spaced. When the vane broadens out again, beyond this narrow neck, these lines are less closely but very regularly spaced. Assuming that the distance between successive lines is a measure of the daily growth of the feather, the setting off of distal appendage or lobe from the rest of the feather was correlated with a period of diminished growth velocity due to a cause not definitely known in this case, but very possibly a nutritional deficiency covering about fifteen days.

Riddle (1908*a*) interpreted in a similar way the relation between down and succeeding definitive feathers. Jones (1907) had shown that the first down and its succeeding definitive feather are produced by one continuous growth, the former being the plumulaceous tip of the latter. But the down feather is differentiated from the pennaceous feather, with which it is continuous, by a short quill, which Riddle (1908) regarded as a defective region due to the same general cause responsible for fault bars, namely, inadequacy of nutrition. True quills also, Riddle thought, resulted from a diminished food supply.

There are, then, certain points of resemblances between these phenomena which, whether normal or abnormal, have been interpreted as results of variations in the food supply, and the phenomena we have described as results of thyroid feeding. There are, however, certain significant points of difference.

Thyroid feeding shortens the vane of the first rectrix in chicks and increases the proportionate length of the calamus by the suppression of proximal barbs and their associated barbules. This resembles effects attributed to poor nutrition.

Thyroid feeding tends to maintain continuity between first and second generations of rectrices. We have no evidence that poor nutrition does this.

Thyroid feeding tends to accelerate the differentiation and eruption of the second rectrices. Poor nutrition, on the contrary, tends to retard these processes.

It appears then that these results of thyroid feeding are of two opposing sorts, only one of which is comparable with the

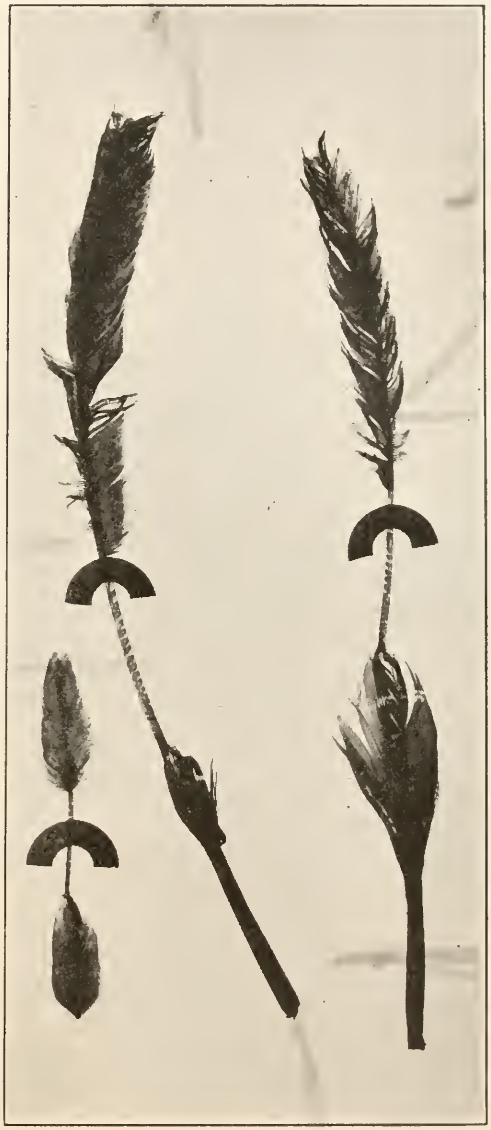

FIG. 3. Three wing feather from young Brown Leghorns showing continuity of feathers in succeeding generations as a result of thyroid feeding. Only a portion of each second generation feather is shown (below).

effects of nutritive deficiency. Such effects vary in intensity with the dose of thyroid administered. The "quilling" in the birds recorded in Table I., more pronounced than in the birds of Table II., followed a heavier dosage of thyroid. And after the exceptionally large daily ration of 1 gram of dessicated thyroid one of our adult males already mentioned replaced 3 pairs of moulted remiges with feathers the proximal portions of whose vanes presented the deformities that characteristically follow starvation. There were pigmentation defects also, but these will be considered at another time.

The second consequence of thyroid feeding, namely, the acceleration of feather differentiation and eruption, would seem to involve an acceleration of division processes in the cells of the feather germ. According to Champy (1922) thyroid added for twenty-four hours to water containing frog tadpoles accelerates cell division in certain zones and tissues far beyond normal velocities. Ebeling (1924), using much more satisfactory methods, has established the accelerating effect on cell division of substances secreted by living thyroid which is added to cultures of fibroblasts *in vitro*. Whether thyroxin would act similarly is not yet known. Thyroxin (Squibb's) does not accelerate the division rate of *Paramecium*, but, on the contrary, in dilutions of 1 : 1,000,000 actually depresses it (Riddle and Torrey, 1923; Torrey, 1923, 1924; Torrey, Riddle and Brodie, 1925). The evidence from our present material will be offered at a later date.

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March 12, 1924.

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ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION.

VI. The Action of Acids.

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In recent years the rôle of acidity and alkalinity in biological processes has been the subject of numerous investigations. The impetus to this field of research was given by the invention of methods for determining the true hydrogen ion concentration of biological fluids and materials. As a result of the great mass of work accumulating along this line of research, there prevails among biologists the impression or opinion that hydrogen ion concentration is of tremendous importance in the life of organisms. Yet, looking back upon the history of science, one may be pardoned for a certain degree of skepticism. Biologists appear prone to attach undue significance to whatever field of investigation happens to be the fashion of the decade and time alone can assign a mass of research on one particular topic to its proper place.

The present series of experiments was undertaken in part as a test of the proposition that hydrogen ion concentration is of fundamental importance in physiological processes. The consumption of oxygen in respiration was chosen as a physiological process essential to the organism in the highest degree and readily susceptible to quantitative measurement. An attempt was made to determine: (*a*) whether increasing the acidity of the environment of an organism has any effect upon the rate of oxygen consumption; (*b*) whether this effect is assignable to the free hydrogen ions or to some other factor. The experiments prove that alterations in external acidity markedly affect the rate of respiratory metabolism of animals; but they also show that the free hydrogen ions are little, if at all, responsible for the observed effect.

Literature.

Surprisingly enough, very few investigations have been carried out on the effect of increased acidity on the respiratory metabolism of organisms. A rather careful search through the iterature has revealed only the following researches.

Among plants, only the lower forms have been investigated. Brooks ('21, '22) measured the carbon dioxide production of *Bacillus butyricus*, *B. subtilis*, and *B. tuberculosis* at various hydrogen ion concentrations. The bacteria were tested in dextrose solutions made in distilled water and the desired acidity was obtained with sulphuric acid. The rate of carbon dioxide production was at the maximum at pH 7.0 for *B. butyricus*, at pH 6.8 for *B. subtilis*, and was decreased at all acidities greater than these. In the case of *B. tuberculosis*, however, the carbon dioxide production was constant at all acidities between pH 7.4 and 4.4 and was decreased only at acidities greater than 4.4. Gustafson ('20) studied the effect of altered acidity on the respiration of the mold *Penicillium chrysogenum*. Variations in acidity between pH 8.0 and pH 4.0 were without effect upon the rate of carbon dioxide production. In acidities ranging from pH 4.0 to 2.65, the carbon dioxide output first rose and then fell; below 1.95 an irreversible decrease in the output was observed. The oxygen consumption was measured at pH 2.0 and was found to be markedly increased over the normal, thus agreeing with the initial carbon dioxide production at this particular acidity. In Gustafson's experiments, the mold was tested in dextrose solutions in distilled water and sulphuric and phosphoric acids were employed to increase the acidity.

Among animals, a few researches are available on eggs, tissues, and intact animals. Loeb and Wasteneys ('11) found that the oxygen consumption of sea-urchin eggs is slightly decreased in sea-water acidified to pH 6.0 to 4.0, and considerably decreased at or below pH 4.0. Thunberg ('09, '10) has carried out a large number of experiments on the action of acids on the rate of oxygen consumption and carbon dioxide production of surviving frog's muscle. The tests were performed in salt solution. Thunberg unfortunately did not determine the pH of his solutions—this procedure was not customary at that time—but gives the concentration of acid in terms of molar strength. Hydrochloric acid decreased both oxygen consumption and carbon dioxide output of the muscle markedly, the decrease varying from 15 per cent. in 1/200 mol. HCl to 72 per cent. in 1/20 mol. HCl. Thunberg also tested the action on gaseous exchange of the frog's muscle of a large number of organic acids, including mono-, di-, tri-, and oxycarboxylic acids of both the paraffin and olefin series. Each acid was tested in the following concentrations: 1/100, 1/25, 1/10, and 1/5 molecular strengths. In the majority of the acids, both the oxygen consumption and carbon dioxide production were decreased at all concentrations but not very greatly so. The maximum decrease with the strongest concentrations was 15–25 per cent. In some of the acids (*e.g.*, propionic, butyric, lactic), the respiratory rate was increased in the more dilute concentrations, decreased in the stronger solutions. In fumaric, malic, and citric acids, the carbon dioxide output was greatly increased, while the oxygen consumption was but little altered. On the other hand, oxalic and malonic acids were found to decrease the carbon dioxide output to a much greater extent than the oxygen consumption. Succinic acid alone decreased the former while increasing the latter. It must be remarked that the concentrations used by Thunberg are extremely high and it is difficult for me to believe that the muscle could have remained uninjured in any of the solutions employed by Thunberg, even though each experiment lasted for only thirty minutes. Exposure to 1/50 or 1/20 mol. HCl, for instance, concentrations used by Thunberg, is, I believe, rapidly fatal to any living tissue. Thunberg does not consider the possibility of injury to the muscle in his experiments and makes no statements concerning the condition of the muscle during or after the exposure. I do not believe his experiments can be accepted as of physiological significance. Gray ('24) has studied the action of acids on the oxygen consumption and activity of ciliated tissues of bivalves. Acetic, butyric, and hydrochloric acids were used, presumably in sea-water. In the acidified water, both ciliary activity and oxygen consumption were diminished and in relation to the amount of acidity. Acetic acid at a pH of 4.6 to 4.2 and butyric acid at 4.2 caused a very great decrease and even complete abolition of oxygen consumption. Hydrochloric acid was less effective than either of the two organic acids, producing only 50 per cent. depression at pH 3.0.

There is a dearth of experiments on entire animals. Jewell ('20) tested the carbon dioxide output of frog tadpoles in distilled water acidified with HCl. The output was decreased to 92 per cent. of the normal at pH 7.0, to 84 per cent. at pH 5.4, to 53 per cent. at 3.8, and to 37 per cent. at 3.2. Powers ('23) studied the effect of carbon dioxide tension on the oxygen consumption of the herring. He found the maximum oxygen consumption at pH 7.6 to 7.8; alterations in the carbon dioxide tension, and hence pH, either above or below this value, lead to a decrease, rather slight, however, in the amount of oxygen consumed by the fish. Powers ('22) is of the opinion that the ability of fish to utilize oxygen depends to some extent on the pH of the water in which they are living.

In addition to such direct measurements of the effect of increased acidity of the medium on respiratory exchange there is available a considerable number of researches on the action of acids on processes in which respiratory metabolism is undoubtedly of great importance. Acidification of the medium has a general retarding or depressing effect on such processes. I can refer here only briefly to this literature. It is a well known fact, attested by many researches, that acidification of the medium retards the cleavage and development of the egg and produces abnormal types of larvæ (cf. Loeb, '98, Moore, Roaf, and Whitley, '05, Child, '16, Medes, '17, and Smith and Clowes, '24, for echinoderm eggs; Child, '25, for hydroids; Child, '17, for annelids; Loeb, '98, and Hall, quoted by Shelford, '23, for teleost eggs; and Hall, '24, and Bellamy, '19 and '22, on amphibian development). The retarding effect of acids on regeneration was noted by Jewell ('20). Acidification of the medium constitutes a general method for altering and controlling polarities (cf. Child, '23, and Rustia, '25).

For a general summary of the rôle of acids in the behavior and life of aquatic organisms the excellent paper of Shelford ('23) should be consulted. I am unable however to agree with Shelford that hydrogen ion concentration is of greater importance than carbon dioxide content for aquatic organisms. The facts that Jewell ('22) found an extensive and varied fauna in an acid stream (pH 5.8 to 7.1) and Jewell and Brown ('24), several fishes living in an acid lake (pH 4.4) indicate that hydrogen ion concentration is not of paramount importance in the distribution of aquatic animals. Allee ('23) noted a lack of relation of hydrogen ion concentration to the distribution of marine animals.

From the investigations cited the general conclusion is certainly justifiable that the acidification of the medium to the proper extent retards or depresses biological processes and activities. My own experiments are in agreement with this general result. But without further analysis the conclusion should not be drawn that the observed effect is assignable to the free hydrogen ions of the acidified medium. Natural waters usually contain salts which undergo chemical changes when the water is acidified. In particular, carbon dioxide is evolved. Unless it is definitely proved that the evolution of carbon dioxide or other secondary chemical changes attendant on acidification of natural waters is not concerned in the result, the observed effect should not be assigned to the hydrogen ion concentration *per se.* Even ordinary distilled water contains some carbonates and other salts which may affect the result. Some of the researches cited in the foregoing review cannot be criticized on this score, or only to a slight extent, as distilled water was used, but in other cases, where natural waters were employed, the carbon dioxide evolved was probably of more consequence in the result observed than the hydrogen ion concentration.

In an extensive study of the depressing effect of acidification of natural waters on the oxygen consumption of aquatic animals, presented in this paper, it is shown that in all probability, carbon dioxide is chiefly responsible for the effect.

Methods.

The general course of procedure in the experiments to be reported was as follows. The rate of oxygen consumption of the animals in normal water was determined for a definite period of time, generally one hour. The normal water was then acidified to the desired extent and the oxygen consumption in this acidified water for one or more successive periods of time determined. The animals were placed in Erlenmeyer flasks, if small, or in wide-mouthed salt bottles, if large, of about 500 cc. capacity. The water to be used was thoroughly aërated and placed in a large elevated receptacle. From this it was siphoned into the flasks or bottles containing the animals and allowed to flow out at the top for a few minutes. The flasks or bottles were then tightly closed and kept in a water bath at constant temperature for the desired length of time, generally one hour. After thoroughly shaking the contents, a sample of 125–150 cc. was then drawn by siphon and analyzed for oxygen content by Winkler's method. Blanks of the water used were of course also drawn at the beginning of the experiment and allowed to stand in the water bath with the animals until the end of the test. The difference between the oxygen content of these blanks and the samples drawn from the flasks or bottles containing the animals gives, after suitable calculation, the amount of oxygen consumed by the animals in the time selected. Immediately after drawing the normal samples, the acidified water was added in the same way and the animals allowed to respire in the acidified water for the same length of time. This was repeated as many times as desired, using the same animals and the same degree of acidification in any one series of experiments. The water was freshly acidified and aërated for each of these determinations. There is no possibility that alterations in the oxygen content of the water in any way affected the result, as the oxygen content was at saturation at the beginning of each test, and the test continued only long enough for the animals to use up a small part of the oxygen present.

The hydrogen ion concentration was determined by means of indicators purchased from the La Motte Chemical Products Company. A set of standard tubes covering the range from pH 3.0 to pH 8.4, with indicators brom phenol blue, brom cresol green, brom cresol purple, and phenol red, was employed. Solutions of these indicators were obtained from the same company. A few drops of the appropriate indicator added to 10 cc. of the water to be tested yields a color which can be compared with that of the standard tubes; the approximate pH is thus easily obtained. Brom cresol green was not found to be reliable and consequently pH 5.0 could not be determined very exactly.

The acids employed were: hydrochloric, nitric, sulphuric, carbonic, acetic, butryic, citric, and tartaric. Each of these acids was used in the following concentrations, in terms of pH: 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, and in some cases 4.5. At least three experiments at each concentration of each acid were performed and in many cases a greater number of trials was deemed necessary. It will be seen that the amount of labor involved was very great and this fact must serve as my excuse for any deficiencies in the investigation which may suggest themselves to the reader.

Experiments with Marine Animals.

The experiments recorded in this section were performed in 1922 at the Hopkins Marine Station, Pacific Grove, California. The animals used were two species of starfish, *Leptasterias equalis*, and *Patiria miniata*, and the nudibranch, *Anisodoris nobilis*. It may be noted in passing that starfish and nudibranchs have been found to constitute very favorable material for respiratory experiments.

The animals were placed in wide-mouthed salt bottles and the tests carried out as outlined above. *Leptasterias* is a small species and several individuals were therefore used in each bottle. As the other two species are larger, generally only one individual was necessary. Generally two successive trials of the oxygen consumption in normal sea-water were carried out. The sea-water was then acidified to the desired amount with hydrochloric acid and a determination immediately carried out. The animals were then usually allowed to stand for two to several hours in the acidified sea-water; another test was then performed. The animals then remained in the acid water over night, about 13 to 15 hours in different experiments, whereupon a third test of the respiration in the acidified water was performed. The animals were then returned to normal sea-water, and after several hours the oxygen consumption was again tested in normal sea-water.

It should be understood that for each test freshly aërated and freshly acidified water was used. The water was brought in directly from the ocean as the sea-water coming through the pipes into the laboratory was found to be toxic. In acidifying the water, concentrated HCl was regarded as a 10 mol. solution and sufficient of this was added to a measured quantity of sea-water to give theoretically a certain molecular concentration of hydrochloric acid. Actually, of course, owing to the salts in the sea-water, the concentration obtained is less than that given in the tables. The hydrogen ion concentrations of these solutions could not be determined as I did not have with me the necessary outfit. It may be roughly estimated, however, that 1/1000 HCl in sea-water is about pH 7.0, 1/800 about pH 6.5, 1/600 about pH 6.0, and 1/400 between 5.0 and 5.5. When allowed to stand, of course, such solutions become continuously more alkaline, owing to the escape of carbon dioxide. For this reason, the solution was not made until a few minutes before it was to be used in the tests. The pH of normal sea-water is about 8.2.

The acidification of the sea-water has little or no noticeable effect upon the behavior of the animals in the lower concentrations, 1/1000 mol. or less. In concentrations of 1/800 mol. or greater, the animals are markedly affected. The starfish withdraw their tube feet into the ambulacral groove. The rays are retroflexed towards the aboral surface. In this position the animals remain during the exposure to acid and are apparently unable to move about or to cling with the tube feet. Nudibranchs display a similar behavior. In the stronger solutions they lose their ability to hold to the substratum with the foot and float about in the water in a state of immobility. The gills however remain expanded and it is not believed that differences in the degree of expansion of these organs are to any extent responsible for the depressing effect of acid on the oxygen consumption. In brief, acidification of the water appears to paralyze the neuro-muscular apparatus and to reduce the animal to a state of forced immobility. Recovery is prompt and complete upon return to normal sea-water. None of the concentrations used had any injurious effects upon the animals.

The results of the experiments are given in part in Tables I and II. A typical experiment for each concentration of acid used for one of the starfish species (*Patiria miniata*) and for the nudibranch is presented in Table I. Three trials of each concen-

Table I.

Effect of the Acidification of the Sea-water with Hydrochloric Acid on the Rate of Oxygen Consumption of the Starfish *Patiria miniata*, and the Nudibranch *Anisodoris nobilis*.

Oxygen consumed given in cubic centimeters, per ninety minutes for the starfish, per hour for the nudibranch. Each vertical column constitutes one experiment, showing successive tests of the oxygen consumption of the same animals or animal. The concentration of the acid is given in molecular strengths.

Starfish.				Nudibranch.			
Oxygen Consumption in Normal Sea-water.							
.43	.40	.31	.34	.78	1.02	.68	.83
Oxygen Consumption in Sea-water Acidified with HCl.							
1/1000	1/800	1/600	1/400	1/1500	1/1000	1/800	1/600
Immediate Effect.							
.41	.32	.23	.22	.76	.77	.49	.50
Effect after 2 to 6 Hours' Exposure.							
.35	.31	.21	.22	.74	.78	.57	
Effect after 13 to 15 Hours' Exposure.							
.42	.34			.74	.75		
Recovery on Return to Normal Sea-water.							
.42	.44	.32	.34	.76	.97	.72	
Initial per cent. Depression.							
5	20	26	36	3	25	28	40

Table II.

Average Initial per cent. Depression of All Experiments on Marine Animals.

Conc. HCl.	1/1500	1/1000	1/800	1/600	1/400
Leptasterias		31	38	38	51
Patiria		9	21	28	34
Anisodoris	7	30	26	31	

tration were made but only one of these is given in the table, and may be taken as typical of the three. The results on the other species of starfish (*Leptasterias equalis*) being in no wise different from those on Patiria are omitted for the sake of economy of space. In Table II. is given a summary of all of the experiments, the three trials with each concentration being averaged. The percentage of depression is figured from the oxygen consumption during the first exposure to the acid.

These experiments, although somewhat preliminary in nature, nevertheless exemplify the action of acids on respiratory metabolism. The conclusions which they furnish were verified by later more extensive work. These conclusions may be briefly stated at this point:

1. Acidification of the medium lowers the rate of oxygen consumption of animals.

2. This depression is greater the greater the degree of acidification up to a certain point.

3. Low concentrations are, however, relatively more effective than high concentrations.

4. After a certain degree of acidification has been attained, further acidification does not greatly increase the amount of depression of the oxygen consumption. The maximum depression obtainable is about 50 per cent.

5. The depressing effect of acids on respiratory metabolism is completely reversible, providing the concentration of acid employed is not actually injurious to the tissues of the animal.

Experiments with *Planaria* in Unaltered Fresh Water.

The experiments to be reported in the remainder of the paper were performed at Chicago and occupied the greater part of my time from October, 1923, to February, 1925. The aim of the experiments was to test the effect upon the oxygen consumption of a fresh water animal of the acidification of fresh water with various acids at a variety of hydrogen ion concentrations. The animal selected for this purpose was the flatworm, *Planaria dorotocephala*. It was chosen for a variety of reasons: it is very abundant, is easily kept under laboratory conditions, has no calcareous parts which might be affected by acid, can be kept quiet during respiratory work, and finally had already served as material for a large amount of physiological experiment. I had originally intended to work also with fish and crayfish but the experiments on *Planaria* required a much greater expenditure of time than I had anticipated and I did not feel justified in spending any more time on the matter. I was further informed by Dr. E. B. Powers that he was already engaged in a similar investigation on fish, and I was quite willing to relinquish this particular task to him.

1. *General Procedure.*—There is little to add to the procedure already outlined. Large stocks of *Planaria dorotocephala* are always at hand in our laboratory. Only stocks which had sojourned in the laboratory for at least one or two months were employed, as the basal respiratory rate of freshly collected material is variable. Only worms which had starved from four days to two weeks were used, since during this period the respiratory rate is constant. Each lot of worms to be used for an experiment consisted of a number of individuals sufficient to consume a readily measurable amount of oxygen in an hour. Such a lot was placed in a 500 cc. Erlenmeyer flask at least several hours before the experiment because when the worms are placed in any clean strange container they are apt to travel about restlessly for some time. By placing the worms in advance in the container in which they are to be tested and by darkening them during the experiment, movement can be practically entirely eliminated. It is certain that movement does not play any rôle in the results. Three such lots of worms were carried throughout the work in order that three tests of each strength of each acid could be performed simultaneously. This procedure shortens considerably the amount of labor involved. Each lot of worms was kept in a particular flask for nearly two weeks and used several times during that period. If desired such lots could then be fed once or twice in the flasks and after four days again utilized for experiments. This procedure was found most suitable because different lots of worms are apt to yield different percentages of depression with the same concentration of the same acid. To obtain a graded set of results showing a graded increase in the percentage of depression with increasing concentration of acid, it is almost necessary to test all of the concentrations of any one acid on the same lot of worms. If different lots are interpolated in such a series, the relation between depression and concentration is less regular, although always exhibiting the same general trend.

The remainder of the procedure was as already described. It may be repeated once more that for each hour's test freshly aërated water was employed and this was freshly acidified to the desired degree a few minutes before being used for each hour's determination. Each test lasted for one hour. A determination of the normal rate of oxygen consumption in normal water (pH 8.0) was first run, and this was followed without pause by a determination in the acidified water. In many cases there were two or three trials in the acidified water, usually with intervals between the trials. During such intervals the animals remained of course in the acidified water. As it was found by repeated experiment that the maximum percentage of depression is obtained on the initial exposure to the acid, these later tests in acid were omitted in the latter part of the investigation. Generally the investigation of each acid was begun with the lowest concentration (pH 7.5) and the concentration increased by steps of 0.5 pH at each succeeding experiment. As will be discussed later this procedure gives the most regular results and great irregularities are introduced if the strongest concentrations are employed first.

There is not the slightest possibility that any differences in the oxygen content of the water either at the start of each test or produced during the test by the withdrawal from the water of oxygen by the animals in any way affects the results. The oxygen consumption of *Planaria dorotocephala* is entirely independent of the oxygen content of the water at all concentrations between 8 and 2 cc. per liter. Whether it is affected by concentrations above or below these limits has not been determined. Suffice it to say that in none of the experiments here recorded nor in any that I have reported in the past with this species has the oxygen content of the water ever reached a value which could have affected to the slightest degree the rate of oxygen consumption of the animals.

The acids used and the hydrogen ion concentrations at which they were tested have already been stated. Concentrations which might have injured the animals during the exposure were avoided. For this reason very few tests were run at acidities greater than pH 5.0. It was desired to keep within physiological concentrations.

All of the experiments on *Planaria* were performed at a temperature of 20° C. and the oxygen in all cases is calculated for this temperature. The results are given in cubic centimeters of oxygen consumed per hour. The animals were not weighed as the oxygen consumed per gram is of no importance for the present experiments. For the benefit of those who may be curious regarding this matter it may be stated that the normal rate of oxygen consumption of this species under the conditions of the experiments (worms of medium size, four to fourteen days after feeding, temperature 20° C.) is about .20 cc. of oxygen per gram per hour.

2. *Water.*—The water used in the experiments comes from a well driven into the ground from the floor of the basement of the laboratory. This water when it emerges from the taps is supersaturated with air and also contains a considerable amount of free carbon dioxide. It has a pH of about 7.3. On account of the gas content the water is heated but not boiled and then allowed to cool overnight before being used for experimental purposes. Water so treated has a pH of 8.0 to 8.2. This is the water regarded in the experiments as "normal" and the respiration in such water is taken as "normal." The water is of course thoroughly aërated before use, bringing the oxygen content up to 6 to 8 cc. per liter.

TABLE III.

ANALYSIS OF THE WATER USED IN THE EXPERIMENTS ON *Planaria*.

All figures represent parts per million. Analysis made in 1922.

Determinations as		Hypothetical combinations.	
Iron—Fe	0.2	Potassium nitrate	1.4
Manganese—Mn	0.1	Potassium chloride	1.1
Silica—SiO_2	7.8	Sodium chloride	7.6
Nonvolatile	0.7	Ammonium chloride	0.1
Alumina—Al_2O_3	1.6	Magnesium chloride	1.0
Calcium—Ca	49.2	Magnesium sulphate	22.1
Magnesium—Mg	16.7	Magnesium carbonate	41.5
Ammonia—NH_4	0.03	Calcium carbonate	123.0
Sodium—Na	3.0	Silica—SiO_2	7.8
Potassium—K	1.1	Alumina—Al_2O_3	1.6
Sulphate—SO_4	17.7	Iron oxide—Fe_2O_3	0.3
Nitrate—NO_3	0.9		
Chloride—Cl	6.0		
Alkalinity (methyl orange)	170		
Residue	193		

The water used is thus the ground water of the Chicago region. It is high in salt content, particularly in carbonates. This water has been analyzed for us by the State Water Survey Division of Illinois. It seems desirable to present the analysis at this point.

3. *Behavior of Planaria in Acid.*—When the water is acidified no alteration in the behavior of the worms is noticeable until a certain degree of acidification is reached. This degree is different for different acids, but is generally in the neighborhood of pH 5.5 to 5.0. The animals lose the ability to glide about, owing apparently to a paralysis of the ciliary mechanism. They exhibit continuous writhing movements, and secrete a large amount of mucus. With greater acidification, they lose ability to cling to the glass and fall to the bottom of the container, where they remain, often with slight writhing movements. They are, when this condition is attained, extremely elongated and generally more or less curved, the ventral surface being concave, the dorsal convex. In short, as in the case of the marine animals, a sufficient degree of acidity induces a paralysis of the motor mechanism, which appears to involve the cilia first, the muscles later.

4. *Experiments with Hydrochloric Acid.*—A large number of experiments, about seventy, were performed with this acid. The normal respiration in normal water (pH 8.0) for one hour was first determined. The water was then acidified to the desired degree and the rate of oxygen consumption in the acid water immediately tested. An interval of one hour in the acidified water was then passed, another determination made in freshly acidified water, another hour passed, and a third determination carried out. It will thus be evident that the respiration during the first, third, and fifth hours in the acid was tested. This was the general procedure in the earlier part of the work. The acidity ranged from pH 7.5 to 4.5 at 0.5 pH intervals. It seems unnecessary to present in detail the mass of data thus accumulated as all of the experiments gave the same general result. A set of typical determinations of the action of hydrochloric acid at different hydrogen ion concentrations is given in Table IV. The averages of all of the experiments performed with this acid are summarized in Table VI. The action is graphed in Fig. 1.

TABLE IV.

ACTION OF HYDROCHLORIC AND SULPHURIC ACIDS AT DIFFERENT HYDROGEN ION CONCENTRATIONS ON THE OXYGEN CONSUMPTION OF *Planaria.*

All figures represent cc. of oxygen consumed per hour at 20° C. Each vertical column constitutes one experiment, giving successive determinations on the same lot of worms.

Normal Respiration, pH 8.0.													
	.42	.44	.37	.48	.34	.34	.42	.43	.38	.40	.50	.58	.43
Respiration in Acidified Water.													
	Hydrochloric.							Sulphuric.					
pH..........	7.5	7.0	6.5	6.0	5.5	5.0	4.5	7.5	7.0	6.5	6.0	5.5	5.0
1st hr.........	.36	.34	.28	.34	.23	.20	.23	.36	.27	.27	.33	.37	.24
3d hr.........	.38	.37	.33	.37	.24	.22	.25	.38	.30	.34	.39		
5th hr........	.38	.33	.30	.38	.27	.20		.34	.34	.26	.43		
Per cent. Initial Depression.													
	13	23	25	29	33	40	46	16	29	35	34	37	45
Recovery after Return to Normal Water.													
	.44	.41	.41	.41	.34	.32	.35	.49	.39	.41	.44	.49	.41

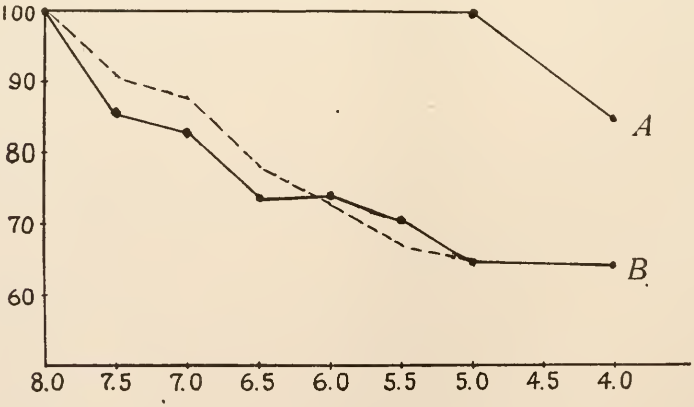

FIG. 1. Graph of the action of acids on the rate of oxygen consumption. Curve *A*, action of hydrochloric acid in carbonate-free water. Curve *B*, action of hydrochloric acid in ordinary water. Dashed line, average curve of the depressing action, obtained by averaging all of the acids except butyric. Percentage of depression, normal respiration being taken as 100, on the ordinate, pH on the abscissa.

It will be perceived by examining these tables that the rate of oxygen consumption of *Planaria* is decreased by acidifying the water with hydrochloric acid and that the percentage of depression is greater the higher the acidity. Yet it must be remarked at this point that the percentage of depression obtained with any given concentration of acid is subject to inexplicable variation even on the same lot of worms and quite commonly when different lots of worms are employed. In most cases this variation did not exceed 10 per cent. but it may reach 20 to 30 per cent., in the higher concentrations. On account of these puzzling variations I was frequently compelled to repeat the experiments many more times than had been anticipated as necessary. The matter of variation is considered at more length later and a possible cause is suggested.

TABLE V.

ACTION OF CARBONIC AND ACETIC ACIDS AT DIFFERENT HYDROGEN ION CONCENTRATIONS ON THE OXYGEN CONSUMPTION OF *Planaria.*

All figures represent cc. of oxygen consumed per hour at 20° C. Each vertical column constitutes one experiment, giving successive determinations on the same lot of worms.

Normal Respiration, pH 8.0.												
	.34	.21	.31	.29	.28	.25	.40	.50	.50	.36	.51	.71
Respiration in Acidified Water.												
	Acetic Acid.						Carbonic Acid.					
pH	7.5	7.0	6.5	6.0	5.5	5.0	7.5	7.0	6.5	6.0	5.5	5.2
1st hr.	.30	.26	.28	.24	.20	.14	.44	.46	.39	.27	.35	.43
3d hr.	.36	.26	.37	.28	.25	.14		.45		.25		
5th hr.	.31		.32									
Per cent. Initial Depression.												
	12	+23	10	18	29	44	+10	8	22	25	32	40
Recovery after Return to Normal Water.												
	.36	.20	.29	.28	.31		.41	.50	.46	.44	.52	

5. *Experiments with Sulphuric and Nitric Acids.*—The action of these two mineral acids was very similar to that of hydrochloric acid. The percentage of depression induced was consistently slightly greater at the same hydrogen ion concentrations

than that produced by HCl. Some detailed data on sulphuric acid are given in Table IV. and the results of all experiments with nitric and sulphuric acids are summarized in the general table, Table VI.

TABLE VI.

SUMMARY OF ALL EXPERIMENTS WITH ALL ACIDS, GIVING THE AVERAGE PERCENTAGE OF DEPRESSION ON INITIAL EXPOSURE TO EACH ACID AT EACH pH USED.

pH.	7.5	7.0	6.5	6.0	5.5	5.0	4.5	3.8
Hydrochloric	14	17	26	26	29	35	45	36
Sulphuric	21	22	27	33	33	34		
Nitric	18	26	28	30	40	40		
Carbonic	+ 2	3	20	21	31	45		
Acetic	6	+ 8	10	31	30	40[1]		
Butyric	16	7	15	7	8	37[1]		
Tartaric	13	14	22	21	31	33		
Citric	+ 4	10	18	31	40	29		

6. *Experiments with Carbonic Acid.*—Carbon dioxide gas was passed through the water until the desired acidity was obtained. In this procedure great care was necessary to avoid depriving the water of its oxygen, since when a gas bubbles through a liquid it removes other gases. The water had to be vigorously shaken with air after the carbon dioxide had been added. A good deal of manipulation is required when working with higher concentrations of carbon dioxide to secure the proper amount of oxygen and carbon dioxide simultaneously in the water. In all of the experiments reported the oxygen content of the water was ample. It may be noted in passing that a combination of low oxygen with high carbon dioxide has a much greater depressing effect than either of these conditions separately. Under ordinary conditions the rate of oxygen consumption is normal in water containing 2 cc. per liter but oxygen consumption is almost abolished in such water if it be saturated with carbon dioxide. When plenty of oxygen is present, the depression produced by saturated carbon dioxide is only about 50 per cent.

Most of the experiments with carbon dioxide are reported in Table V. In the more dilute solutions—pH 7.5 to 7.0—carbon dioxide acidity produces less depression than do other mineral acids. In fact, in these concentrations, there is a tendency towards stimulation of the respiratory rate. Out of nine experiments, stimulations of 2 to 12 per cent. were obtained in five cases. The amount of stimulation, however, lies within the experimental variation. The truth probably is that carbon dioxide acidity does not affect the rate of oxygen consumption until the acidity reaches a greater degree than pH 7.0. At greater acidities than pH 7.0, the rate of oxygen consumption is depressed and to a degree similar to that produced by other mineral acids. It was not possible to obtain an acidity with carbon dioxide greater than pH 5.2 with the well water, owing probably to the buffers present in the water.

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[1] Animals injured.

7. *Experiments with Acetic and Butyric Acids.*—These two acids were selected as examples of the paraffin acids, supposed to penetrate organisms readily. It was anticipated that the depressing effect of these acids would be greater than that of mineral acids. Such, however, was not the case, but the contrary result appeared. Acetic acid, in acidities between 7.8 and 7.0 tended to stimulate the rate of oxygen consumption. Of six experiments performed at pH 7.5, the rate of oxygen consumption was unaffected in two cases, stimulated in two, and depressed in the remaining two. Of six experiments at pH 7.0, all but one were stimulating. It seems probable that low concentrations of acetic acid accelerate the rate of oxygen consumption. At concentrations greater than pH 7.0, all concentrations depressed the rate of oxygen consumption during the first hour's exposure. During later exposures, a tendency toward acceleration was again manifest. These facts are brought out in Table V. and VI. Butyric acid was the least effective in inducing depression of all of the acids used. This result was very surprising and still remains inexplicable. Butyric acid has only a slight depressing action at all concentrations between pH 7.5 and 5.5. At pH 5.0 a depression of 30 to 40 per cent. appears but this concentration of butyric acid is lethal and the worms begin to die within an hour. Acetic acid at pH 5.0 is also injurious. The data on butyric acid are summarized in Table VI., which gives the initial depression induced. Later exposures did not alter the result.

8. *Experiments with Tartaric and Citric Acids.*—The former was

selected as an example of a dibasic, the latter of a tribasic acid. The results were not of particular interest and are summarized in the general table, Table VI. The action of tartaric acid was very similar to that of the mineral acids. Citric acid was less effective than the inorganic acids at concentrations lower than pH 7.0.

9. *Consideration of Other Factors.*—(*a*) *Size.* It was found long ago by Child that young (small) individuals are more susceptible to lethal concentrations of various substances than are old (large) individuals of a given species. MacArthur ('20) studied the relation between age (size) and susceptibility to acids in *Planaria dorotocephala.* He found that in concentrations of hydrochloric and other acids which are quickly lethal, between pH 2.0 and 4.5, the young (small) individuals die sooner than the old (large) ones. At slightly lower concentrations, pH 4.7 to 4.9 for HCl, the relation is reversed, the larger individuals succumbing first. In still weaker solutions, planarians live indefinitely. These results have been repeatedly confirmed in this laboratory in class work, although of course the precise concentrations required to yield the results mentioned are subject to variation, owing chiefly to differences in the physiological condition of the worms themselves.

Since according to these results, small worms are more susceptible to acids than large worms at concentrations greater than pH 4.5, it seems probable that the percentage of depression induced by acids should bear some relation to size. This was tested in a number of experiments, in which the depressing action of HCl on large and on small worms was compared. Only recently collected material was used as the metabolic differences between worms of different sizes are greater in such material. The heads of all worms were removed before the test, in order to eliminate movement, as small worms are more active than large ones. Decapitated worms are very inactive. The small worms used were under 10 mm. in length, the large ones over 20 mm. Twelve experiments were performed at concentrations of HCl ranging from pH 3.6 to 4.6. In ten of these the percentage of depression was greater in the small than in the large worms. The difference ranged from 3 to 20 per cent. In two cases the result was reversed, the larger worms exhibiting about 10 per cent. more depression than the small ones. In general, then, the experiments indicate that in concentrations which will kill within a few hours and in which small worms die slightly faster than large worms, the small worms are more greatly depressed by the acid. It should be added that the animals suffered no injury during the period of exposure and recovered completely.

(*b*) *Previous History with Respect to Acid.*—From my experiments I have gained the distinct impression that the amount of depression induced by exposure to acidified water is to some extent dependent on the previous history with respect to acid. If a given lot of worms, which has never been exposed to acidified water, is tested at rather high acidity, say pH 5.0, it is commonly found that the amount of depression is much less than would ordinarily be expected at that acidity. If on the other hand, such worms are gradually accustomed to acidified water, by exposing them first to pH 7.5 and gradually increasing the acidity, then a much greater percentage of depression is obtained at high acidities. One of the most striking cases of this kind in my investigation occurred in working with sulphuric acid. Three lots of worms, never before exposed to acidified water, were tested in water acidified to pH 5.0 with sulphuric acid. The percentages of depression obtained were 20, 30, and 15 respectively. The same lots of worms were then exposed on successive days to various concentrations of sulphuric and hydrochloric acid, beginning at 7.5 and gradually working down to pH 5.0 again. When sulphuric acid pH 5.0 was then again tried, on the same worms, the depressions obtained were respectively 39, 56, and 45 per cent. The same type of result was frequently met with. It appears that when worms are suddenly exposed to rather strongly acidified water, they are able to produce or manufacture some substance which protects them from the acid. But after repeated exposures to acidified water, the substance is either exhausted or the worms have become so accustomed to residing in acidified water that they no longer respond to it by producing the substance. Sudden exposure to acid may act as a stimulus to production of basic substances; repeated exposure fails to stimulate.

It seems probable that in this behavior of the worms is to be found the explanation of the numerous puzzling variations in the degree of depression obtained.

(*c*) *Acclimation.*—It was my intention at the beginning of this work to determine whether the animals could recover from the depression induced by acid if allowed to remain for some time in the acidified solution. I found, however, that the experiment is impractical, because of the carbonate content of the water used. When the water is acidified it soon becomes alkaline and unless acidified to a point which would be fatal to the worms, returns to an alkaline condition within 24 hours. Consequently if worms are placed in the acidified water at a certain pH, the water does not remain at this pH, but the pH rises (the acidity falls). If then the worms are tested at the new pH, the depression is naturally less than it was at the beginning of the exposure. If tested at the original pH, the depression is greater than at the pH attained by the standing solution. In brief, it is impossible to determine the effect of long continued exposure to a given pH, unless the water is freshly acidified and changed every hour, for two or three days. As this is physically impossible, for me at least, the experiment had to be abandoned. I have, however, a number of experiments, especially with HCl, in which the oxygen consumption was tested during the first, third, and fifth hours of continuous exposure to a given concentration. Some of these data appear in Table IV. In general it was found that there is very slight if any recovery during successive hours of exposure. After this had been repeatedly determined, the tests of the later exposures were abandoned.

The reader will no doubt at once inquire why carbonate-free water was not employed in a study of acclimation. The reason for this as will appear later is that acidified carbonate-free water does not affect the oxygen consumption of Planaria.

10. *General Results and Discussion.*—We may now state and discuss the results obtained with acidified unaltered water.

(*a*) Acidification of the water generally causes a depression of the rate of oxygen consumption. This was true of the three mineral acids, hydrochloric, sulphuric, and nitric, at all concentrations employed. Even a change from the normal pH 8.0 of the water to 7.8 by addition of such acids induces a measurable lowering of the rate of oxygen consumption. In the case of some acids, notably acetic, less so with carbonic, there was some tendency to an acceleration or stimulation of the oxygen consumption at acidities between 7.8 and 7.0. Yet this effect was so slight, and the variability of the results so great that little emphasis can be placed upon this finding, unless a very large number of experiments were carried out. All of the acids used caused depression at all acidities greater than pH 7.0, but the action of butyric was very slight, almost nil.

(*b*) The percentage of depression increases with increasing acidity within certain limits.

(*c*) The lower concentrations are, however, relatively more effective than the higher concentrations. This is generally the case wherever chemicals are applied to living organisms. The general form of the curve obtained is seen in Fig. 1. This type of curve is so commonly obtained in physiological experimentation that it must possess some deep significance. I am unable however to suggest any explanation of this type of curve.

(*d*) The inorganic acids, except carbonic, are in general more effective than the organic, particularly in the lower concentrations, pH 7.5 to 6.5. This finding was the contrary of my expectations and contrary to the results of Gray ('24). It is generally believed that organic acids, such as acetic and butyric, penetrate protoplasm readily, while mineral acids are unable to do so. Obviously one would then expect the organic acids to act more powerfully on respiratory metabolism than the inorganic acids. As this was not found to be the case, it must be concluded that the penetrability of the acid has no bearing on the result. The lack of action of butyric acid remains inexplicable.

(*e*) The hydrogen ion concentration cannot be the chief cause of the depression induced, because different acids do not produce the same percentage of depression at the same pH. The result does not substantiate the contentions of Loeb ('22) that the action of acids on colloids depends only on valence and hydrogen ion concentration. According to Loeb's ideas, all of the monovalent acids, such as hydrochloric, nitric, acetic, and butyric should have produced the same amount of depression at the same hydrogen ion concentration. Reference to Table VI. shows that this is not at all the case, butyric acid furnishing a notable exception. According to Loeb also, di- and tri-basic acids should be less effective than monobasic. This again is not upheld by my results. Sulphuric acid is even more effective than hydrochloric, although being dibasic it should be only half as effective. Tartaric, another dibasic acid, has about the same efficiency as the monobasic mineral acids. Of course, it is not certain that Loeb intended his ideas to apply to living organisms. Further the hydrogen ion concentrations with which Loeb worked are mostly instantly or rapidly fatal to living organisms. It remains to be proved whether the statements of Loeb will hold at physiological concentrations of acid. I have been informed that Michaelis has publicly stated that they do not hold and that the Hofmeister series remains unshaken. From the fact that in my experiments different acids produce different degrees of depression at the same hydrogen ion concentration it appears necessary to conclude that the hydrogen ion concentration is not the principal factor in the result.

(*f*) When a certain degree of acidification has been attained, further acidification does not increase the percentage of depression. The percentage of depression obtained at pH 5.0 is about the maximum that can be produced without actual injury to the animals. It appears that a depression of 50 per cent. is the most that can be obtained with any acid on the average. Of course individual experiments may yield a depression slightly greater than this. A depression of 58 per cent. is the greatest recorded in the dozens of experiments performed with the various acids and this figure was obtained on young worms. The statements in this paragraph apply only when an ample supply of oxygen is present in the water.

(*g*) The decrease in the rate of oxygen consumption reaches its maximum value for any particular concentration during the first hour of exposure to the acidified water. Prolonged exposure does not increase the depressing effect. On the contrary there is generally some slight rise in the oxygen consumption during several hours exposure. This is too small however to be considered of significance.

(*h*) The depressing action of acids is completely and promptly reversible, wherever an actual injury to the tissues of the animal has been avoided. Recovery occurs almost immediately, commonly within the first hour after return to normal water. Several experiments were devised to test the possibility that the decreased oxygen consumption while in the acid might be compensated for by an increase over the normal during the period immediately upon return to normal water. In these experiments the oxygen consumption was first tested in normal water, then in acidified water, of a concentration to give at least 30 per cent. depression, then immediately in normal water again. In most of these cases the respiration had risen to the normal value during the first hour after return to normal water. In a few cases, the oxygen consumption was below normal. In no case was any rise over the normal figure observed.

(*i*) The experiments justify the use of acids as agents for experimentally producing a state of depression. They also substantiate the generally held view that the effects produced by acids on such processes as cleavage, development, and regeneration are assignable to a reduction in the rate of respiratory metabolism.

Experiments with *Planaria* in Carbonate-free Fresh Water.

The experiments had reached the point outlined above by the summer of 1924 and I intended to bring the investigation to a close. A number of matters puzzled me greatly but I was unable to devise any means of throwing further light on them. At about this time, however, my attention was drawn to the experiments of Clowes and his associates ('23, '24) in which it was shown that the carbon dioxide set free by acidification of sea-water markedly influences the result and is in some cases the real agent involved. At first I was not inclined to believe that carbon dioxide was responsible for the results which I had obtained with acids. It seemed to me that if carbon dioxide were chiefly or wholly responsible for the observed effects, carbon dioxide acidity should be more effective than acidities produced by other acids and the action of various acids should be similar at the same hydrogen ion concentrations. As these conditions did not obtain in my experiments, I considered it unlikely that the findings of Clowes applied to them. It seemed necessary to me, however, that the matter should be tested by experiment. A new series of experiments was therefore begun in the fall of 1924 using carbonate-free water. The results obtained convinced me that the depressing action of acidified water is largely due to the carbon dioxide liberated in it.

In connection with these experiments a large number of trials in ordinary carbonate-containing water were carried out as controls. These constituted repetitions of the work of the previous year. It was very puzzling to me to find that all acids were less effective than had been the case in the preceding year. This was particularly noticeable at the lower concentrations, pH 7.5 to 7.0. The mineral acids produced about the same depressing effect as previously at the higher concentrations, 6.5 to 5.0. The organic acids (acetic and butyric were the only ones tried) were also markedly less effective than had previously been the case, at all concentrations tried. I am unable to explain this state of affairs except on the assumption that the carbonate content of the water had decreased in the meantime. The fact that in many cases the controls gave a smaller percentage of depression than previously made it difficult to arrive at conclusive results.

1. *Preparation of Carbonate-free Water.*—Carbonate-free water was prepared according to the method of Smith and Clowes ('24*b*). Two cc. of concentrated hydrochloric acid were added to eight liters of the well water in a large bottle. Air from the compressed air system was passed through the water in the bottle for 24 hours or more. This treatment was found to remove the carbonate from the water completely. Several times liter samples of this water were evaporated to dryness and the residue tested for carbonate with entirely negative results. In some cases borax was added to the carbonate-free water to serve as buffer but as the results were not affected by this procedure, it was generally omitted. The oxygen consumption of the worms in this carbonate-free water, with or without borax, was repeatedly compared with that in the normal unaltered well water. No difference was found.

2. *Action of Mineral Acids Added to Carbonate-free Water on the Rate of Oxygen Consumption.*—The same amount of acid was added to the water as would be required to produce the desired pH in unaltered water. Alkali was then added until the pH in question had been attained. Rather extensive experiments were performed with hydrochloric acid, less extensive with sulphuric and nitric acids. The results in all three acids were the same and were very striking. Acidification of carbonate-free water with these acids has *absolutely no effect* on the rate of oxygen consumption of Planaria at all concentrations between pH 7.5 and 5.0. At pH 4.0, a slight depressing effect was noted, about 15 per cent. This result is graphed in Fig. 1.

It is thus proved that practically the entire depressing effect on the oxygen consumption of *Planaria* produced by the addition of mineral acids to natural water arises from the carbon dioxide set free in such water by decomposition of its contained carbonates by the acid added. The depressing effect is thus a carbon dioxide depression. It is probable that the carbon dioxide penetrates the animal in the gaseous state and acts within the animal as such or by inducing an internal acidity (cf. Jacobs, '20).

3. *Action of Organic Acids in Carbonate-free Water.*—Only acetic and butyric were tried. I was particularly interested in testing these two acids as they are believed to penetrate organisms readily. The result should serve to indicate whether the efficiency of carbon dioxide is merely a question of penetrability. Unfortunately decisive results could not be secured, owing to the fact, already mentioned, that very little depressing effect was obtained in the controls. Butyric acid, previously found to be the least effective of all of the acids tried, was in this series of experiments quite ineffective in normal water. Even at pH 5.0, an injurious concentration, no depression of the oxygen consumption in normal water appeared. Consequently the action of butyric acid in carbonate-free water could not be determined. With acetic acid, some results were secured. Acetic acid was about half as effective in inducing depression as in the experiments of the preceding year. At concentrations weaker than pH 7.0 there were again indications of acceleration of the rate of oxygen consumption in the controls. At acidities of pH 6.5

or greater, depression was produced in normal water, but to a less extent than previously. In the same worms, in carbonate-free water acidified with acetic acid, the depression induced was noticeably less than in the controls. It therefore follows that the depressing action of acetic acid is also in large part due to the carbon dioxide which is sets free. It appears that the penetrating powers of carbon dioxide do not entirely explain its difference from other acids.

4. *Action of Carbon Dioxide Added to Carbonate-free Water.*—These experiments were designed as a sort of crucial test of the proposition that carbon dioxide is the cause of the depression induced by acidification of natural waters. The proposition was upheld in the most striking manner. The addition of carbon dioxide gas to the same pH causes the same degree of depression, whether added to normal, or to carbonate-free water. It thus appears to be reasonably certain that the depression of the rate of oxygen consumption in acidified water is caused chiefly by carbon dioxide.

The question then arises: Is the amount of carbon dioxide liberated in carbonate-containing water by the addition thereto of acids, different with different acids at the same hydrogen ion concentration? It became necessary to determine the actual amount of free carbon dioxide present when the well water was acidified with various acids.

5. *Determinations of the Amount of Carbon Dioxide Liberated on Acidification of the Unaltered Water.*—The normal well water, pH 8.0, was acidified to the desired pH with various acids. A sample of 100 cc. was then immediately drawn and the free carbon dioxide in it determined by titration with $N/50$ barium hydroxide, properly protected from the carbon dioxide of the air, using phenolphthalein as indicator.

Hydrochloric, sulphuric, acetic, and butyric acids were investigated at ranges of pH 7.5 to 4.0. It was found that the amounts of carbon dioxide liberated from the carbonates of the water by different acids are similar, but not identical, at the same pH. Such differences probably account for some of the different percentages of depression obtained with different acids at the same pH. But butyric acid was also found to liberate nearly as much carbon dioxide at the same pH as does hydrochloric acid. The lack of depressing action of butyric acid therefore remains at present inexplicable.

Nearly all of the carbonate of the water is liberated as carbon dioxide at a pH of 5.0 and all of it between pH 5.0 and 4.0. The amount of free carbon dioxide present at these acidities is about 30 cc. per liter. At first the fact that all of the available carbonate in the water is decomposed at pH 5.0 to 4.0 appeared to explain the result that the maximum percentage of depression is obtained at such acidity and further acidification does not increase the depression. But the additional fact discussed in the next paragraph that increasing the amount of carbon dioxide by adding the gas is not effective in intensifying the depression invalidates the suggested explanation.

Analyses were also made of the amount of carbon dioxide gas required to produce a certain pH. It was found that more of the gas is present in the water at a given pH than is set free in the water by adding other acids to the same pH. This result would be expected, since it is highly probable that when the water is acidified, considerable time must elapse before all of the acid reacts with the carbonate of the water. Consequently immediately after acidification part of the free hydrogen ions present are derived from the acid added, and not from the reaction of carbon dioxide with water. The difference in carbon dioxide content between water acidified with carbon dioxide gas and water acidified by other acids is small at pH 7.0 or even 6.5 but at pH 6.0 there is three times and at pH 5.0 nearly ten times as much carbon dioxide gas in the water as is liberated by mineral acids at those same hydrogen ion concentrations. These results are shown in Fig. 2.

We are thus faced with the question—why is carbon dioxide not more effective as a depressing agent than any other acid, particularly at the higher acidities? There was to be sure evidence that carbon dioxide at pH 5.0 for instance has a greater depressing action than any other acid tried. Yet the difference between it and other acids is not as great as might be expected. It becomes necessary to assume, in view of the facts at hand, that the amount of depression of the respiratory rate which can be induced in Planaria by carbon dioxide is limited and does not exceed 50 per cent. as long as the oxygen supply remains ample. If the oxygen supply is reduced, although not to a point where the respiration rate would be affected under ordinary conditions, the oxygen consumption can be nearly abolished if the water is saturated or nearly so with carbon dioxide. A carbon dioxide content of 30 cc. per liter appears to yield the maximum depressing effect, about 50 per cent., that can be obtained with carbon dioxide, when the oxygen supply is adequate.

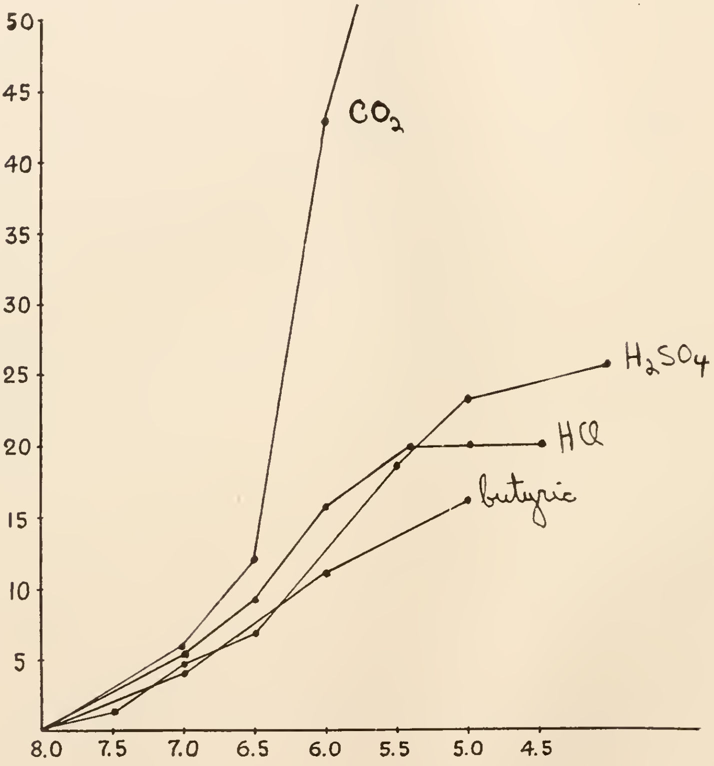

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FIG. 2. Graph of the amount of carbon dioxide liberated in the well water by various acids. Amount of carbon dioxide in cc. per liter on the ordinate, pH on the abscissa. The amount of carbon dioxide gas required to produce a given pH is also shown.

6. *Conclusions Concerning the Experiments with Carbonate-free Water.*—(*a*) The depressing effect of acids on the oxygen consumption of Planaria is almost wholly abolished when carbonate-free water is used, except when the acidity is produced by carbon dioxide gas.

(*b*) An acidity due to carbon dioxide gas is equally depressing in ordinary and in carbonate-free water.

(*c*) From the statements in (*a*) and (*b*) it is concluded that the depressing action of acids on oxygen consumption is almost wholly due to the carbon dioxide liberated on acidification of natural waters.

(*d*) Different acids at the same hydrogen ion concentration do not immediately liberate the same amounts of carbon dioxide from the natural carbonate-containing water. This accounts in large part for the fact that the depressing action of different acids is not the same in degree at the same pH.

(*e*) All of the carbonate of the water used is decomposed at a pH of 4.0 to 5.0, giving a carbon dioxide content of about 3 per cent.

(*f*) The lack of action of butyric acid is not explained, for this acid also liberates carbon dioxide from the carbonates of the water. Possibly butyric acid in some way prevents the penetration of carbon dioxide into the animals.

(*g*) The amount of carbon dioxide gas required to produce a given pH is considerably greater at acidities of more than pH 6.5 than is the amount of carbon dioxide liberated in the water by other acids at the same pH. One would therefore expect that CO_2 would be much more effective as a depressing agent at acidities from 6.5 to 5.0 than any other acids. This was not noticeably the case. The difficulty could be explained by assuming that the depressing action of CO_2 reaches a maximum at a concentration of 3 per cent. and is not altered by further increase in the concentration of the gas.

(*h*) Since the depressing effect of acids is due largely to carbon dioxide and since this gas is an end product of metabolism, it becomes readily understood that organisms or parts must be affected by acids in relation to their respiratory metabolism. The higher the rate of the latter, the greater would be the depression induced by acids.

Relative Toxicity of Different Acids.

An attempt was made to discover the hydrogen ion concentration at which each of the acids used would cause the death of *Planaria* within a relatively short period of time, say, two to three hours. A sort of trial and error procedure is necessary to determine this matter. The results are not very exact but the relative toxicities of the different acids were plain enough after a few trials. Butyric acid is by far the most toxic, as found also by other investigators, and kills the animals within two or three hours at pH 5.0. Acetic acid ranks next in toxicity, killing in the time specified at pH 4.4. Tartaric and citric acids come next, killing within two or three hours at 3.6 and 3.4 respectively. The three mineral acids are nearly equally toxic, sulphuric being slightly more effective, killing in two or three hours at pH 3.2 to 3.4, while hydrochloric and nitric acids must be used at pH 3.0 to 3.2 to obtain the same result. The order of toxicity may then be expressed as follows: butyric > acetic > tartaric > citric > sulphuric > nitric = hydrochloric.

Carbon dioxide at saturation (about pH 5.2) was not found to kill the animals as long as the oxygen supply was adequate.

The death of the animals took place with equal rapidity and at the same hydrogen ion concentrations in both normal and carbonate-free water. This proves that the death is not due to the carbon dioxide set free by acids in normal water but is a direct acid effect. This is further evidenced by differences in the appearance and in the manner of disintegration of the dead animals. Death in acids appears to be generally due to coagulation. This was also noted by Mrs. Lewis, '23.

In view of the fact that the death of the animals results from a direct coagulating action of the acids while the depression of the respiratory rate by acids is caused almost wholly by carbon dioxide, no relation would be expected between the toxicity of different acids and their depressing action. This is the case. Butyric acid, the most toxic, is in non-lethal doses the poorest depressant. The experiments do not of course serve to show the amount of depression at or near the death point, except in the cases of acetic and butyric acids, pH 5.0, Table VI. Since different acids are equally lethal at different hydrogen ion concentrations, it follows that the free hydrogen ions cannot be the chief factor in toxicity. The penetrating powers of the acid are probably of great importance and the nature of the anion or molecule may also be involved. Butyric and acetic acids which probably penetrate organisms the most readily of all the acids employed are also the most toxic. In a study of the toxicity of various acids for ciliate Protozoa, Collett ('19) reached the conclusion that hydrogen ion concentration is not the most important factor.

SUMMARY.

1. The effect of acidification of the medium on the rate of oxygen consumption of aquatic organisms was studied.

2. The acids used were: hydrochloric, nitric, sulphuric, carbonic, butyric, acetic, citric, and tartaric. They were added to water to produce acidities ranging from pH 7.5 to 5.0, at intervals of 0.5 pH.

3. *Planaria dorotocephala* was the chief animal used as material. Some tests with hydrochloric acid were also made using starfish and nudibranchs.

4. The acidification of natural waters, either salt or fresh, (pH 8.0), by any of the acids used except butyric causes a decrease in the rate of oxygen consumption at all acidities greater than pH 7.0. The majority of acids also cause depression between 7.8 and 7.0 but in the case of acetic acid and more doubtfully carbonic, there was some tendency towards a slight acceleration of the rate of oxygen consumption at these lower concentrations.

5. The decrease in the rate of oxygen consumption due to acids is completely and promptly reversible, as long as the animals are not actually injured.

6. The acidification of fresh water from which all carbonates have been previously removed has no or only a slight effect upon the rate of oxygen consumption of *Planaria*, except when the acidity is produced by carbon dioxide.

7. The depressing action of carbon dioxide is the same whether the gas is added to ordinary or to carbonate-free water.

8. From 6 and 7 it follows that the depressing action of acids in natural waters is due chiefly or wholly to the carbon dioxide which they liberate from the carbonates of such waters.

9. The depressing action of acidified natural waters on the rate of oxygen consumption of *Planaria* is not the same with different acids at the same hydrogen ion concentration. This appears to be due largely to the fact that the amount of carbon dioxide immediately liberated from the carbonates of the water differs with different acids at the same pH.

10. The depressing action of acids in natural waters is greater the greater the acidity up to an acidity of about pH 5.0. This is due to the fact that the more acid added, the greater is the quantity of carbon dioxide liberated.

11. Lower concentrations are, however, relatively more effective than higher ones.

12. The maximum amount of depression of oxygen consumption that can be induced by acids is about 50 per cent. This occurs at pH 4.0 to 5.0 and further acidification of the water does not increase the percentage of depression. At the acidity at which the maximum depression appears, the carbon dioxide content is about 3 per cent.

13. All of the carbonates of the fresh water employed are decomposed by acids at a pH of 4.0 to 5.0, producing a carbon dioxide concentration of 3 per cent. This might explain the facts given in 12 were it not that concentrations of carbon dioxide gas much higher than 3 per cent. do not increase the percentage of depression beyond 50 per cent., as long as the oxygen supply is ample. Concentrations of carbon dioxide gas up to 25 per cent. were tested.

14. From the facts cited in 13 it appears necessary to assume that the depression of the rate of oxygen consumption which can be induced by carbon dioxide does not exceed 50 per cent. as long as the oxygen supply is adequate.

15. A combination of high carbon dioxide content and low oxygen content practically abolishes the oxygen consumption of *Planaria*, even though the oxygen content used (2 cc. per liter) would be ample for normal respiration in the absence of carbon dioxide.

16. No explanation has been discovered for the differences between the action of carbon dioxide and other acids at low concentrations (pH 7.5 and 7.0).

17. Butyric acid has almost no action on the oxygen consumption of *Planaria*. No explanation has been discovered for this fact.

18. The different acids employed are lethal for *Planaria* in a given arbitrarily selected time (2 to 3 hours) at different hydrogen ion concentrations. The order of toxicity and the hydrogen ion concentrations at which the acids are equally lethal are: butyric (5.0), acetic (4.4), tartaric (3.6), citric (3.4), sulphuric (3.2) and nitric and hydrochloric (3.0).

19. The facts given in 18 prove that the hydrogen ion is not the cause of death but either the anion or the molecule of the acid is involved. Penetrability is probably also a factor. Death in acids appears to be due to coagulation.

20. The order of toxicity of the acids and the pH at which they are equally lethal are the same in ordinary and in carbonate-free water, showing that death is not due to carbon dioxide liberated.

21. Acidification of natural waters constitutes a method for depressing the rate of oxygen consumption of aquatic animals for experimental purposes.

22. The experiments herein presented cast doubt on the supposed importance of hydrogen ion concentration *per se* in biological processes.

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BIOLOGICAL BULLETIN

THE RELATION OF *HERPETOMONAS ELMASSIANI* (MIGONE) TO ITS PLANT AND INSECT HOSTS.

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Herpetomonas elmassiani (Migone) is at present the only species of latex-inhabiting herpetomonad known in the United States. Its plant host is the common milkweed, *Asclepias syriaca* L., in the latex of which it has been found in great numbers in Maryland and New Jersey (Holmes, 1924, 1925*a*). Its suspected insect host in these locations is a red and black hemipterous insect, *Oncopeltus fasciatus* (Dall.). The same species of plant flagellate appears to inhabit other milkweeds in countries along the Atlantic coast of Central and South America. It has been reported from Haiti, Honduras and Paraguay, and doubtless exists at intermediate points between these countries and the locations in the United States. In southern locations other species of *Oncopeltus* frequenting the infected plants have seemed to act as insect hosts.

In view of the lack of apparent pathogenicity of the herpetomonads in Maryland milkweeds (Holmes, 1925*b*), it seems desirable to report upon the relation of the flagellate to the tissues of its hosts. The well known species, *Herpetomonas davidi* (Lafont), which inhabits the latex cells of Euphorbias in Europe and elsewhere, is pathogenic to its host (França, 1914, Nieschulz, 1922), in which it causes modifications of the latex cells and neighboring portions of the plant sufficient to stunt or kill whole branches or even whole plants. The reasons for the lack of harm resulting in the milkweed host from the presence of *Herpetomonas elmassiani* (Migone) may be the freedom from infection of some of the latex systems even of heavily infected plants, the entire freedom from penetration of tissues aside from the latex systems, and the sufficient food supply presented to the flagellates by the milky vacuole fluid itself in the latex cells.

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[1] Joint contribution from the laboratories of the Boyce Thompson Institute for Plant Research and of the Department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University.

The fact is often overlooked that plant flagellates of the type of *Herpetomonas davidi* (Lafont) and *Herpetomonas elmassiani* (Migone) have been found *only* in such plants as have abundant latex. Those engaged in research in this field are of course acquainted with this restriction of the range of the organisms, and take it into account in most of their work, but do not always seem to have it very definitely in mind. Others whose interest in plant flagellates arises from some other, less immediate, source are often entirely unmindful of the situation until it is brought to their attention.

To the present time no acceptable species of flagellates of the genera *Herpetomonas*, *Leishmania*, *Crithidia* or *Trypanosoma* have been found in plants other than those provided with a milky juice or latex.

Perhaps the situation would be better understood if it were commonly known that latex does not occur extracellularly in plants, but intracellularly. Thus the flagellates which are transferred by their insect hosts to the latex cells of plants are not to be found thereafter at random in the plant tissues, but are strictly intracellular (not intracytoplasmic) parasites.

A description of the cells containing the latex will make clear the relation of any latex-inhabiting organisms to the host plants.

The latex ducts of the plants with which protozoölogical studies have been most concerned are those known as simple ducts, because they do not fuse with each other in the course of their wanderings. Among the Asclepiadaceæ, Euphorbiaceæ, Apocynaceæ and Urticaceæ the cells destined to become the latex ducts of the mature plants are already distinguishable in the embryo. Their nuclei divide again and again, and the cells elongate tremendously and branch repeatedly, but no cross walls are formed, nor do the ducts fuse with one another, so that eventually the few original cells penetrate every part of the plant, and still remain distinct and separate from each other. The thin cellulose walls are lined with a layer of cytoplasm containing numerous nuclei. In the vacuole is collected the milky, usually white latex.

It is well to bear in mind that there is another type of latex system among plants, in which the original cells fuse together by the destruction of their partitions and cross-walls. The result of the process is the formation of interlacing vessels, in which the latex is contained. This type of latex system is found in such plants as lettuce.

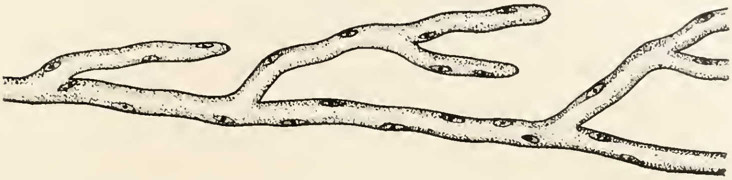

FIG. 1. Diagrammatic representation of a portion of a simple latex duct such as is found in the species of the genus *Asclepias*. The latex is secreted by the wall of cytoplasm into the extensive central vacuole. Such a duct will not fuse with others in the course of its growth.

Diagrams representing these two types of latex ducts are shown in Figs. 1 and 2.

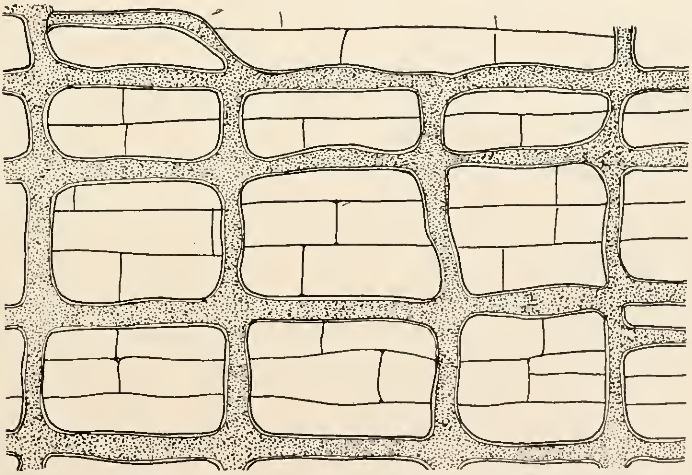

FIG. 2. Diagrammatic representation of a system of latex vessels such as is found in lettuce. The original cells fuse together to form a network, instead of remaining as independent latex ducts. The type of latex cell involved in protozoölogical studies has been that shown in Fig. 1.

The flagellates known to inhabit the latex systems of plants are probably all insect parasites. They enter the cells of the plant hosts only when the insects introduce their beaks directly into the latex to feed. In the cells they do not enter the cytoplasm, so far as can be seen, but inhabit the milky juice which fills the long cell vacuole. There they grow and multiply rapidly, as is indicated by the many dividing specimens present in latex smears from infected plants stained with Wright's stain. The flagellates are shut off from other cells of the plant and even from other latex cells of which there may be several. Unless a single plant were infected several times by insects it is unlikely that all the latex ducts would become infected, even though one or more might.

Localized Infections.

If one latex system of a plant is infected, a macroscopically localized infection may result unless that system happens to penetrate every leaf and flowerlet of the plant.

Early in the season of 1924 a search was made for such cases, for the previous year all the infections had appeared systemic.

It seemed likely that insects might repeatedly bite plants during the late summer, but that during June and early July any infections which might occur from insect carriage would be the result of a minimum number of infective bites.

The first example of the way in which this worked out in the field was met when a plant of a group surveyed thoroughly every few days gave a negative record after showing flagellates on several occasions. The later examinations of this plant showed that flagellates were present, but were not always to be found in the single drops of latex preserved as records. For this plant, then, a new system of sampling was instituted. Samples were taken from ten leaves instead of from one. It was found that some leaves were positive and others negative, just as one might reasonably expect if only a few of the latex cells were parasitized.

The study of sections of petiole and leaf tissue gathered at this time showed the even more interesting fact that in plants never suspected of having localized infections only a few of the latex cells were inhabited by organisms. It was easier to find negative cells than positive in specimens from apparently heavily parasitized plants. Smears from these plants showed very large numbers of organisms, yet when a droplet was taken for examination as much latex must have been contributed by uninfected cells as by infected. In the cells containing the organisms the concentration was so great that a moderate dilution was entirely ineffective in changing the appearance of the drop as stained for examination.

Since the plants above mentioned seemed when in the field to have systemic infections in that the slightest wound in any portion of the leaves or stems gave infected latex, and yet the microscopic examination of sections showed but a few of the cells infected, it is evident that the whole plants were penetrated by a relatively few single cells, and that if each individual latex cell fell short of extending completely throughout its plant, it still must have achieved very nearly this remarkable feat. This type of cell attains a notable total size and contains tremendous numbers of nuclei. Its latex-containing vacuole also is probably nearly if not quite the largest of cell vacuoles, making up in length far more than it lacks in breadth.

Confinement to Latex Cells.

Careful study failed to show any flagellates in the plant tissues outside the vacuoles of the latex cells. In some sections there were indeed abnormal and deceptive appearances caused by the flow of latex by capillary action along the conducting tissues. When pieces were cut to be fixed, latex always flowed out over the wounded surfaces. Since the turgidity of the plant was in part relieved by this loss, other specialized cells such as the spiral vessels were invaded by currents of latex along with which the flagellates themselves were carried. The abnormality of this process was made evident by examining the invaded ducts throughout their entire length. The flagellates were seen to be present only near the cut surfaces and to become less and less crowded as the distance from the exterior increased. The tissues in the interior presented a truer picture of conditions in the living plant.

Since the flagellates were normally confined to the latex cells, and indeed to the *latex* filling the long vacuoles, it became evident that certain observations made in the field were of more significance in their relation to the biology of the flagellate than had at first been realized.

It had been noticed that infections did not spread from plant to plant in groups connected by a common axis. Such plants arose from separate buds, the latex systems of which were independent of each other. In the axis itself no ducts occur, so that there is no chance for wandering through such a connection.

Late in the autumn of 1924 two plants which had been under observation for months were dug up to determine their exact relation to each other. One of these had been consistently negative for flagellates all summer. The other had been as consistently heavily infected in every part above the ground, with the exception of the seeds which are always free from invasion. The two plants were separated from each other by no more than six inches of axis, from which common source they had both sprung as buds. The absence of latex ducts in the axis and the confinement of the flagellates to the latex of the infected plant made it impossible that the nearby negative plant should be invaded except from some outside source of the organisms.

The practical confinement of the insect, *Oncopeltus fasciatus* (Dall.), suspected of being the insect host of the flagellate, to the blossoms and pods of the milkweed plant in feeding is also made significant by histological studies of the latex system, which in these two parts becomes much more prominent than it is in the stems or leaves. The soft tissues under the outer green coverings of the pods contain numerous branches of the latex system, and in the area between the pedicels and the bases of the petals of the flowers the latex ducts are exceedingly close together and voluminous. This offers a favorable feeding location to the insect, and, by reason of the crowded flagellates here in infected plants and the softness of the tissues, opportunity is offered for the infection of insects from the plant and for the infection of previously uninfected plants by the insects.

The Flagellates of *Oncopeltus fasciatus* (Dall.).

It is not yet known with certainty whether *Oncopeltus fasciatus* (Dall.) is the insect which carries the milkweed flagellate from one plant to another. But since there are several reasons for suspecting it to be the carrier, a study of its flagellates was undertaken. All along the coast of America, from Paraguay in the south to New Jersey in the north, wherever the flagellate has been found in the latex of the milkweeds some species of the genus *Oncopeltus* has been found feeding on the infected plants. These insects have always been more characteristic of the particular plants harboring the flagellates than any other type of insect. Just north of New Jersey, where no flagellates are known in the plants, another insect genus *replaces Oncopeltus*. The specimens of this which have been examined thus far have been entirely negative for any flagellate, though infected with Sporozoa both intracellularly and extracellularly in the salivary glands. This coincidence of the range of the milkweed flagellate with the range of *Oncopeltus fasciatus* (Dall.) suggests that the presence of the flagellate is dependent on the presence of the insect. Moreover *Oncopeltus* feeds characteristically on the pods and flowers of the plant, and since plants bearing seed are the ones to which the infection is practically limited it seems even more probable that *Oncopeltus* is the host. Another bit of circumstantial evidence is gained from the study of the morphology of the flagellates from the insect and from the plant. The plant flagellates are characterized by a twist in their ribbon-like bodies. This is rare in insect flagellates, but is found in the case of the parasites of *Oncopeltus*. The insect feeds on latex. The infected plants have swarms of flagellates in *their* latex in every portion of the upper parts of the plants. Thus the insect could easily become infected from his feeding. In the insect the histological studies about to be described have shown swarms of flagellates in definite lobes of the thoracic salivary gland. The secretion of this gland is led by a simple duct directly to the mouth parts during the process of feeding, so that there should be no great mechanical difficulty in the transfer of the insect's flagellates to the interior of the latex ducts of the plant.

It is natural then that in spite of the difficulties which have been experienced up to this time in attempting to obtain plant infections from the insects in regions where plant infections are not known in the field, efforts to establish the relation of the plant and insect flagellates by histological studies have been made. The results have been negative so far as obtaining definite proof of the identity of the two forms is concerned, but such interesting observations have been made during the study that they will be described here.

The first insects to be sectioned and stained were collected during 1923. At first the flagellates were overlooked, and that for two very good reasons. The principal search was made for them in the intestinal tract, where they do not occur in my material, if indeed they ever occur there. And the salivary gland forms, stained as carefully as they may be, never stand out with the clearness by which those in latex smears are characterized. For the process of drying and staining with Wright's stain, though open to the objection that the nuclear detail is lost, gives bright sharp pictures of the organisms, usually making them much darker than the background. This is far from the case with wet-fixed material, sectioned, and stained even with so good a stain as iron hæmatoxylin. By this process the nucleus may be stained with all the desired sharpness, but the background of salivary secretion retains the stain far more than does the flagellate's cytoplasm. So that usually one sees the body of the flagellate only as an unstained area surrounding the nucleus. The wall of the salivary gland also retains the stain so tenaciously that it must usually be left very black that the internal structure of the flagellate may be seen at its best. This makes the whole field very dark, with the object to be examined exceedingly delicate and lightly stained. When these first specimens of *Oncopeltus* were examined a second time for a different purpose, clusters of the flagellates were noted by chance on the wall of the gland. The slide under observation at the time was stained with a mixture of aniline dyes, but the finding was at once confirmed by iron hæmatoxylin slides of the same material.

During 1924 a quantity of material was obtained for sectioning to discover the extent of the infection among the insects during the late season when the plants were becoming more and more widely infected, to determine with certainty that the organ in question was really the salivary gland of the insect and not a salivary receptacle or some other organ, to see whether the organism present had more than one stage in its life-history in the insect, and to find exactly its relation to the salivary gland tissues and secretion.

The study of the morphology of the gland in which the flagellates occurred required complete series of serial sections of three insects. The gland of one of these was reconstructed section by section, for the lobes of the gland cannot be readily visualized from the separate sections. Sometimes but one or two show, at other times there appear to be five or six lobes because of the inclination of the section and the twists in the organ itself.

Histology of the Salivary Gland.

The salivary gland nearly fills the dorsal half of the thorax. Its efferent duct leads away from the point at which the three lobes come together, first running back toward the abdomen immediately under the gland, then turning and running forward. It dips beneath the œsophagus near its junction with the proventriculus, and beneath the ventral chain of nerve ganglia between the sub-œsophageal ganglion and the ganglion immediately posterior to it. From this point the duct runs forward to the mouthparts where its secretion enters the pump or syringe and the hypopharynx.

It was interesting to note that the three parts of the gland could be distinguished readily in iron hæmatoxylin eosin sections by the character of the secretion in each, in spite of the fact that the outline of each might be most deceptively placed according to the plane of the section. The anterior lobe contained frothy material staining pink with eosin. The ventral lobe was distended with a smooth or very slightly granular substance which also stained pink with eosin. It was thus easy to distinguish these two by the consistencies of their contents. The dorsal lobe was remarkable in that its fluid retained some of the iron hæmatoxylin stain and in addition picked up eosin eagerly, nearly attaining the brilliant orange red color characteristic of blood corpuscles in tissues stained for example with Delafield's (not Heidenhain's) hæmatoxylin and eosin.

Localization in Dorsal and Anterior Lobes.

It was in the third or dorsal lobe that the flagellates were really abundant. There they were often massed like a new tissue lining the entire lumen of the gland. The dark stain which the fluid took made it difficult to study the internal structure of the organisms. Yet they were so abundant that some were usually favorably located for observation. The flagellates lined the dorsal lobe most heavily near its posterior extremity. Nearer the efferent duct the organisms were often in groups or clusters, isolated a little from the rest of the mass, but closely bunched together with flagella attached to the wall.

In the anterior, foam-filled lobe there were a few flagellates always when they were present in the dorsal lobe. In the posterior lobe there were none, no matter how heavily the insect was parasitized.

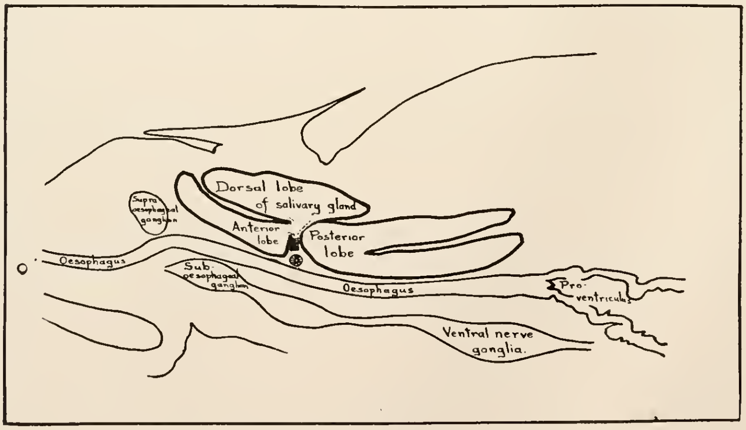
Supra oesophageal ganglion
Dorsal lobe of salivary gland
Anterior lobe
Posterior lobe
Oesophagus
Sub-oesophageal ganglion
Oesophagus
Pro-ventriculus
Ventral nerve gonglia.

Fig. 3. Portion of a longitudinal section through *Oncopeltus*, showing the relative positions of the salivary gland and other thoracic organs. The three lobes of the gland come together in this particular section and the point of entrance of the salivary duct is shown.

That the fluids from the three lobes intermingled slightly at the common point of contact where the three fluids drained into the same efferent duct was indicated by the mixed consistencies and colors just at this exit point. In the smooth orange-red fluid a cloud of pink was always seen to have penetrated for a short distance. In the two pink portions, granular and frothy, red clouds were also visible just at the same point.

Until the gland had been reconstructed by drawing each section on blotting paper, cutting out and pasting together the individual sections, and coating the whole model with a beeswax and paraffin mixture, it was impossible to get an adequate idea of the complete gland. The lobes were so complicated in some planes of the section that it was not certain that no salivary reservoir entered the question. As the work was completed, however, it became evident that the structure in which the flagellates were located was a three-lobed thoracic salivary gland, with a single common exit. A diagram of the longitudinal section through the opening of the three lobes is to be seen in Fig. 3.

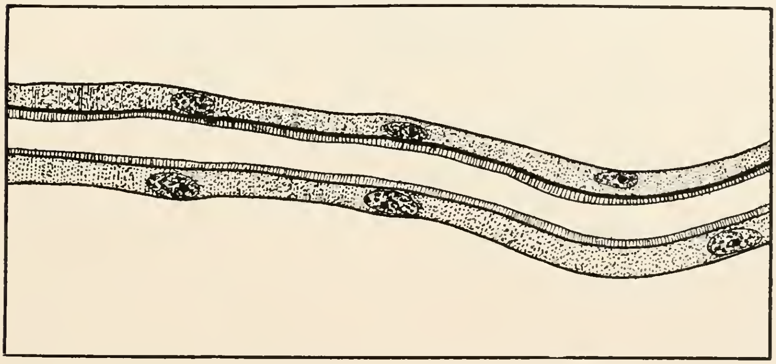

FIG. 4. A portion of the efferent duct of the salivary gland of *Oncopeltus*.

The cells of the duct are characteristic and interesting and with the secreting cells of the gland are so readily recognizable that no difficulty arises in identifying them even in sections which show but a tiny fragment of the salivary apparatus.

The duct is lined with a single layer of cells the nuclei of which are sometimes branched. A portion of the duct is represented in Fig. 4. A similar detailed drawing of the cells of the wall of the salivary gland is shown in Fig. 5. The glands are commonly distended with fluid. This escapes gradually, as required, through the efferent duct.

With the recognition of this organ as a thoracic salivary gland one of the objects of the histological examination of the insects was accomplished.

It was interesting to note that the flagellates always developed in the dorsal lobe most abundantly. A few penetrated the anterior, but none the ventral lobe. Evidently the differences in the compositions of the salivary secretions of the three lobes, indicated at once by the different staining reactions of the three portions, had also a significant effect on the organisms, inducing them to inhabit one portion more than others, and excluding them from the ventral lobe.

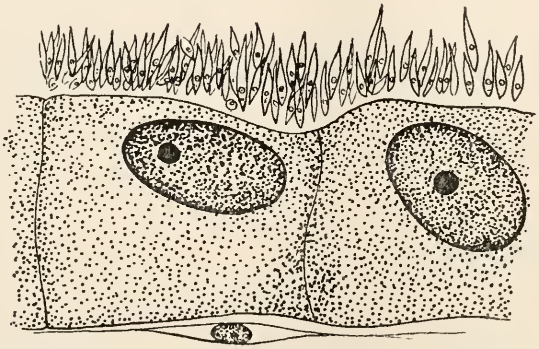

Fig. 5. A portion of the wall of the dorsal lobe of the salivary gland of an infected *Oncopeltus*. The nuclei of the gland cells are gigantic in comparison with the minute nuclei of the flagellates.

In my material no intestinal forms were found. This is remarkable when one considers that the type species of the genus *Herpetomonas* is a strictly intestinal flagellate in the common housefly. Salivary gland forms are the exception rather than the rule among Herpetomonads, and it would be expected that intestinal forms of the flagellate would be more noticeable than the gland forms. But if the flagellates of *Oncopeltus fasciatus* (Dall.) ever show intestinal forms I have not been able to secure the proper material to demonstrate them.

In addition to the complete series of sections of three individuals of *Oncopeltus fasciatus* (Dall.), a group of ten of the same species was sectioned and studied by choosing representative sections for staining and for examination. Sixteen individuals of *Lygæus kalmii* from Massachusetts were treated in the same way.

The object of using *Lygæus* was this: the limit of the range of *Oncopeltus fasciatus* coincides with the limit of the range of the plant flagellate, *Herpetomonas elmassiani* (Migone). But *Lygæus* is a very closely related insect, replacing *Oncopeltus* in the north where the flagellates are not found. It was desired to know whether it also had flagellates in its salivary glands. The thirteen specimens of *Oncopeltus*, which came from Maryland in September, 1924, at a time when the spread of plant flagellates was going on rapidly, were all infected with the exception of three individuals, which were rather young nymphs. The sixteen specimens of *Lygæus* were all negative for flagellates, both in the intestinal tract and in the salivary gland.

In the *Lygæus* examined there *were* infections with sporozoa in the glands, curiously enough in the same lobes frequented by the flagellates of *Oncopeltus*. One of the species present in *Lygæus* was occasionally seen also in *Oncopeltus* along with the flagellates. But in *Lygæus* the infections were much heavier, and decidedly destructive to the gland cells which were penetrated by intracellular stages and often rendered useless for secretion by the growth of the parasites and the consequent death of the cells.

The absence of flagellates in sixteen specimens of *Lygæus* collected at the end of the season was strikingly in contrast with the presence of large numbers of flagellates in the glands of ten of the thirteen *Oncopeltus* sectioned. If *Oncopeltus* is responsible for the spread of the milkweed flagellate it is no wonder that the spread is very rapid in September and early October when so large a percentage of the then rapidly multiplying insects are positive.

Of the original problems for the solution of which the histological work on *Oncopeltus* was carried out, but one remains for consideration. Does the flagellate of *Oncopeltus* have more than one stage of its life history in the insect? It seemed likely at first that there would be developmental stages of the herpetomonad in the intestine. But no colonization of the intestinal tract was indicated by any of my material. All the organisms were in the glands. The question then arose as to the exact position of the parabasal body in the forms colonizing the walls of the salivary gland. Careful study showed that in all cases in which the relative positions of nucleus and parabasal body could be definitely determined, the organism was constantly a herpetomonad. Smears of the insect flagellate confirmed this, for in them the position of the organelles could be determined in every single case far more readily than in sections of the gland. This question is not quite safely settled, for at some other season of the year the evidence might differ from that which I have been able to gather, but with the knowledge of the exact part of the insect parasitized, the dorsal and anterior thirds of the thoracic salivary gland, and the extent of infection during the season at which the insects are busily feeding on the infected plants, it will be easier to work on the questions concerned with the insect host of the flagellate.

Summary.

Histological studies of the milkweed host of the flagellate *Herpetomonas elmassiani* (Migone) showed that the organisms were confined to the latex system, in which they were intracellular but not intracytoplasmic. The latex is secreted into the general cell vacuole of the latex duct, and it is in this that the organisms were found. No other cells or parts of cells were found to be penetrated.

During the early part of the summer one or a very few latex cells in a plant were sometimes infected, for in *Asclepias* the original latex cells of the embryo never fuse. Because of this condition occasional localized infections appeared, in which a few leaves of the infected plant were found to be free from organisms.

The flagellates of *Oncopeltus fasciatus* (Dall.), a red and black hemipterous insect suspected of being the insect host of *H. elmassiani* (Migone), were found to inhabit the three-lobed thoracic salivary gland. In the gland these were definitely localized, colonizing only the dorsal and anterior lobes.

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HEAT PRODUCTION BY THE EGGS OF *ARBACIA PUNCTULATA* DURING FERTILIZATION AND EARLY CLEAVAGE.

CHARLES G. ROGERS AND KENNETH S. COLE.[1]

The general problem of development has in it so much of the unexplained that any attempt to add to our information by attacks from new directions may seem warranted. The work here reported was first undertaken several years ago, but was not carried to a successful issue until this past summer because of experimental difficulties unforeseen at the start.

The work was originally undertaken in an attempt to check the work of Warburg and of Loeb and Wastenys concerning the oxygen consumption of eggs before and after fertilization. It will be recalled that these investigators had found that immediately after fertilization there occurs a remarkable increase in the rate of oxygen consumption, amounting to 4 to 6 times the amount used before fertilization. It is to be assumed that there is also a corresponding increase in the carbon dioxide production of the eggs. This latter would, of course, be exceedingly difficult to measure in the case of marine eggs. If there is any considerable increase in oxygen consumption following fertilization there should be a corresponding increase in the amount of heat produced by the eggs as a result of the oxidation process. The question to be faced was whether, with the facilities at our command, we would be able to make a series of measurements which would bear examination by a physicist. Preliminary tests made in 1920 and 1921 indicated that the production of heat by fertilized eggs was a measurable quantity. These tests, observed in the latter year by a group of physicists who were visiting the Marine Laboratory, proved to be sufficiently encouraging to warrant further expenditures of time and money.

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[1] Contribution from the Zoölogical Laboratory of Oberlin College, and from the Marine Biological Laboratory, Woods Hole, Mass. To the Director and other officials of the Marine Biological Laboratory we express our thanks for many courtesies extended to us.

Since this investigation was first projected others have made studies of a somewhat similar character. Myerhof (6) measured the heat production of segmenting Echinoderm eggs by means of a Beckmann thermometer, using a large vacuum flask for his calorimeter. Certain irregularities that occur in his curves may be due to experimental error, but suggest changes in the rate of heat production at different stages of development. Since then Shearer (7) has carried out similar measurements in connection with his work on the oxidation processes of Echinoderm eggs and finds that the rate of heat production after fertilization is constant for at least ten or twelve hours. He makes the statement that readings were taken at fairly frequent intervals at the commencement of the experiment, and at intervals of several hours after that. In view of this statement it seemed advisable to us to repeat the work making frequent readings and using methods of higher precision.

POSSIBLE SOURCES OF ERROR.

In an investigation involving the measurement of such slight temperature changes as are expected here care must be taken to foresee and provide against all possible heat transfers into or out from the experimental flasks or to know the magnitude of such transfers. In any event such heat losses or gains should be small as compared with the total heat production which it is desired to measure. In our experiments the following possible channels of heat transfer existed, and were checked: Conduction to or from the water in the flasks

(1) by the air in the mouth of the flask,
(2) by means of the glass forming the neck of the flask,
(3) along the main thermopile,
(4) along the secondary thermopile,
(5) along the tubes of the stirring apparatus,
(6) along the fertilizing tube,
(7) by changes in the temperature of the water surrounding the flasks.

There are also possibilities of error arising from lack of care in controlling the conditions environing the electrical apparatus. Among these may be mentioned:

1. Mechanical jarring of the galvanometer used in making the measurements.
2. The temperature of the room must be maintained as nearly constant as possible during the course of an experiment. It was found to be especially necessary to avoid the possibility of drafts of air striking the apparatus.
3. It was found necessary to shield the electrical apparatus from stray electrical currents. This was found to be of the greatest importance following a severe electrical storm.
4. It was found wise to avoid all stress and strain in the wires of the thermopiles, such as might be caused by too much bending of the wires, or placing tension upon them.

It was found possible to obviate much of the possibility of the errors of the first group by using a water cap, designed so as to provide a current of water coursing continuously over the experimental flask as well as around it.

Apparatus and Methods of Work.

The general method employed was that of the micro-calorimeter, developed by Hill in his work upon muscle. Fig. 1 shows the experimental set-up used. This method has one serious disadvantage. Since corrections for heat loss are dependent upon the temperature difference and the time, long runs can not be made, since these corrections soon become a very large part of the result. In this work the corrections could be kept less than 15 per cent. of the total temperature change during a period of two and a half or three hours. Two straight sided commercial vacuum flasks of about 75 cc. capacity were used, (I.) containing 50 cc. of the egg suspension, and (II.) an equal quantity of water. These flasks had been especially exhausted through the kindness of Dr. W. R. Whitney of the Research Laboratory of the General Electric Company. When flask (I.) contained 50 cc. of water it had a heat loss of 16 calories per hour per degree difference in temperature between the interior and the exterior. The flasks used by Hill (2) had a loss of about 12 calories per hour for 250 cc. and those used by Shearer (7) about 19 calories per hour for 800 cc. This small loss for a flask of such small capacity is evidence of the great value of very careful evacuation. The flasks were submerged in running sea water to within a short distance of their tops, and a water cap (*d*) made according to a design by White (9) was placed over them. A pipette (*e*) projected through the cap and was arranged so that "dry sperm" could be held in it, and then mixed with the contents of flask (I.) whenever desired. A continuous flow of water was maintained through this cap so that the whole formed a uniform temperature enclosure. The sea water temperature remained fairly constant except for periods of about four hours after sunrise and sunset. The variation of temperature never exceeded 0.001° C. during the period occupied by an experiment.

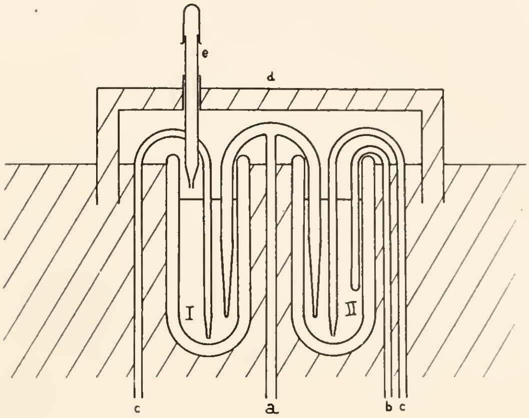

FIG. 1. Diagram of experimental set-up. I. and II., Vacuum flasks. *a*, main thermopile. *b*, auxiliary thermopile. *c*, *c*, stirring tubes. *d*, water cap. *e*, sperm pipette.

STIRRING APPARATUS.

It has been noted by many investigators that eggs of Echinoderms must not be heaped upon each other if normal development is to take place. In our work it was desirable to use as large a number of eggs as possible in order to get the largest possible temperature change. It was therefore necessary to devise some method of stirring the eggs which would render it impossible for them to settle to the bottom of the flask and remain there for any considerable length of time. By the more constant stirring the eggs would at all times be able to get their needed supply of oxygen and to get rid of carbon dioxide. Work previously done by one of us (R.) had shown that stirring the water in the experimental flasks once every two or three minutes by means of an ordinary pipette was sufficient to allow the eggs to go through a normal cleavage. Such stirring was not sufficient to ensure that the water in the flask would be of uniform temperature throughout. It seemed necessary, therefore, to devise some automatic stirring device which would keep the water thoroughly stirred at all times during the course of an experiment. As a matter of interest it was found during the course of the experimentation that a failure of the stirring apparatus for as much as three minutes could be detected by a marked variation in the galvanometer readings.

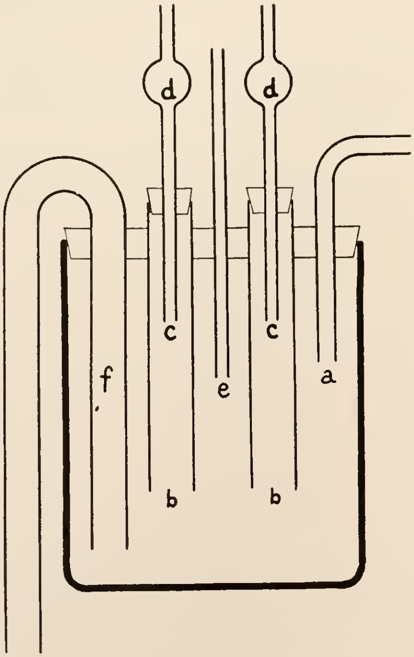

FIG. 2. Stirring apparatus. Details described in text.

The stirring was accomplished as follows: Saturated air was bubbled from the jets (*c*, *c*) at the bottoms of the flasks, by means of the automatic intermittent siphon device shown in Fig. 2. A steady stream of water flowed in at (*a*) and as the water level rose in the bottle, air was trapped in (*b*, *b*) and forced out through (*d*, *d*). When the water reached the lower end of (*e*) it rose in the tubes, but not in the bottle (since the stopper was air tight) until (*f*) was filled beyond the bend. Then (*f*) acted as a siphon and emptied the bottle, allowing (*b*, *b*) to drain, and finally draining itself. Then the process started over again. Short pieces of rubber tubing cut at an angle were slipped over both ends of (*f*) and the lower ends of (*b*, *b*) to help in breaking the meniscus and draining these tubes. The quantity of air sent over each time could be varied by adjusting the height of the small tubes (*c*, *c*) in (*b*, *b*), and the period of the apparatus could be changed by raising or lowering (*e*). The small bulbs (*d*, *d*) prevented drops of water from being forced over into the wash bottles. These wash bottles had inlet tubes of small cross section, and were submerged in the same bath with the flasks. They served to complete the saturation of the air, if it were not already saturated, to bring it to the temperature of the bath before it went into the flasks through (*c*, *c*), and to act as a trap, preventing the suction of liquid out of the flasks through (*c*, *c*) when the stirrer bottle was emptying. In this work 7 cc. of air was sent through each flask every fourteen seconds. During a six-day continuous run, this apparatus did not vary from its average period by more than 2/5 second, always delivering the same and equal quantities of air to the flasks.

Electrical Equipment.

The electrical equipment used in these experiments consisted of the main and secondary thermopiles, galvanometer, potentiometer, storage battery cells, Weston Standard Cell, switches, etc. The main thermopile (*a*, Fig. 1) was composed of twenty pairs of copper-constantan junctions, and gave 733 microvolts per degree Centigrade, by direct calibration. (The constantan wire used was that manufactured by the Driver-Harris Wire Company, and sold under the trade name "Advance.") The reason for the unexpectedly low value of the thermopile was not found. The junctions were enclosed in thin glass tubes filled with naphthalene and the lag of the couple was less than twelve seconds. The auxiliary or secondary thermopile (*b*) had five pairs of junctions and gave the temperature difference between flask II. and the surrounding bath. The e.m.f. developed by the couples was measured by a "White" potentiometer. This instrument, manufactured by Leeds and Northrup, gives dial readings by single microvolts and is so arranged that the resistance in the galvanometer circuit remains constant. This fact makes it possible to read fractions of a microvolt with the galvanometer. In this work one microvolt gave a deflection of 18.5 mm. on the galvanometer scale, so there were about 13,550 mm. per degree. With the high magnification telescope used it was easily possible to estimate fifths of a millimeter on the galvanometer scale. The galvanometer was of the D'Arsonval type, also manufactured by Leeds and Northrup. Its resistance was 13 ohms, period 5 seconds, and sensitivity 10^{-8} amperes per cm. with the scale a little over three meters distant. The total resistance of the galvanometer circuit was 53 ohms, which was an ohm more than the critical damping resistance. In spite of this fact there were never any oscillations, even when the sperm were introduced into the experimental flask,—only what seemed to be a very steady and somewhat rapid rise. The galvanometer circuit was shielded as far as possible, as suggested by White (10) to prevent the entrance of leakage currents into the circuit. It was, however, impossible to shield the thermopiles effectively since their leads were carried through the running sea water and very strange and erratic e.m.f.s were introduced into the circuit if the shielding system for the potentiometer and galvanometer had anything to do with the salt water or any of the piping in the room. Care had to be taken not to use for connections any wire that had been unduly bent or in any other way maltreated so as to destroy its homogeneity. On the whole the electrical apparatus worked very satisfactorily.

It was our original plan to use Hill's ingenious application of vacuum flasks to the twin calorimetric method (2) but it was found that for our small flasks the heat loss did not remain even approximately constant as the volume of the contents was varied. It was also impossible to obtain accurate values for the conduction coefficients from cooling curves for large intervals, since Newton's law of cooling applies to small and constant temperature differences, *i.e.*, equilibrium conditions. This requirement could not be met since there was not available any current measuring device of sufficient accuracy to allow the use of electrical heating. It was necessary therefore, to get the cooling corrections under the actual experimental conditions. Following White (9)

Let θ_1 = temperature of flask (1),
θ_2 = temperature of flask (2),
θ_3 = temperature of external bath.

The temperature coefficient of conduction for the flask is defined as the temperature change between the inside and the outside of the flask in unit time when the temperature difference between the inside and the outside is unity. This may be written in mathematical form for flask (I.), $K_1 =$; and for flask (II.), $K_2 =$.

$$K_1 = \frac{1}{\theta_1 - \theta_3} \cdot \frac{d\theta_1}{dt},$$

$$K_2 = \frac{1}{\theta_2 - \theta_3} \cdot \frac{d\theta_1}{dt}.$$

Similarly, for the heat conduction between the flasks along the thermopile,

$$k_1 = \frac{1}{\theta_1 - \theta_2} \cdot \frac{d\theta_2}{dt},$$

$$k_2 = \frac{1}{\theta_2 - \theta_1} \cdot \frac{d\theta_2}{dt}.$$

Also let w_1 and w_2 equal the temperature changes due to stirring and evaporation. Then considering temperature changes where there is no liberation of heat in either flask

$$\begin{aligned}\frac{d(\theta_1 - \theta_2)}{dt} &= K_1(\theta_1 - \theta_3) - K_2(\theta_2 - \theta_3) + k_1(\theta_1 - \theta_2) \\ &\qquad - k_2(\theta_2 - \theta_1) + w_1 - w_2 \qquad (1) \\ &= (K_1 + k_1 + k_2)(\theta_1 - \theta_2) + (K_1 - K_2)(\theta_2 - \theta_3) \\ &\qquad + w_1 - w_2.\end{aligned}$$

$\theta_1 - \theta_2$ is proportional to θ_a, the e.m.f. of the main thermopile,
$\theta_2 - \theta_3$ is proportional to θ_b, the e.m.f. of the auxiliary thermopile.

So we may write

$$\frac{d\theta_a}{dt} = K_a\theta_a + K_b\theta_b, \tag{2}$$

since both theoretically and experimentally, $w_1 - w_2 = 0$ in (1). If the quantity of heat H is liberated in (1) and the heat capacity (water plus the water equivalent of the flask) is c, then

$$\frac{d\theta_a}{dt} = \frac{1}{c}\frac{dH}{dt} - K_a\theta_a - K_b\theta_b$$

and

$$\begin{aligned} H &= c\left\{ \int_{\theta_0}^{\theta_a} d\theta + K_a \int_{\theta}^{t} \theta_a dt + K_b \int_{\theta}^{t} \theta_b dt \right\} \\ &= c(\theta_a - \theta_b) + c\left\{ K_a \int_{0}^{t} \theta_a dt + K_b \int_{0}^{t} \theta_b dt \right\}, \end{aligned} \tag{3}$$

where θ_0 is the value of θ_a when $t = 0$.

Independent runs were made to determine the values of K_a and K_b. With θ_b small as compared to θ_a, $d\theta_a/dt$ was determined over the range for the values of θ_a used and found to be linear in θ_a. The same was done for θ_b with θ_a small. With 50 cc. in both flasks the values obtained were

$K_a = .0046$ microvolt per minute per microvolt difference,
$K_b = .0014$ microvolt per minute per microvolt difference.

The water equivalent of the flasks was found to be 8 cc. so that $c = 58$ cc.

A TYPICAL EXPERIMENT.

The preparations for an experimental determination of the heat production of the eggs of *Arbacia* involved a variety of considerations not usual in ordinary experimental work in zoölogy. By careful tests the running sea water of the laboratory had been found to be the most satisfactory form of available thermostat, the temperature of the water changing only very slightly during any experimental period—usually only in thousandths of a degree. Care was, therefore, taken to have all glassware and implements used at the temperature of the sea water. Flasks, pipettes, graduates, beakers, finger-bowls, dissecting instruments, wash bottles of the stirring apparatus, thermopiles, water-cap and the animals to be used were all left in running sea water of uniform temperature for some time before the beginning of the experiment. Whenever possible all of the eggs used in an experiment were taken from a single female. These were allowed to stand for a few minutes in a finger bowl in a little more than 50 cc. of sea water. The finger bowl was floated in running sea water. Remnants of the ovary and other debris were removed from the finger bowl by forceps or pipette. After a few minutes the eggs were stirred in the water so as to be evenly distributed throughout the whole mass of water and exactly 50 cc. of the suspension was transferred to flask (I.) by means of a volumetric pipette. Also 50 cc. of sea water were placed in flask (II.). The temperature of flask (II.) was then made enough higher than that of flask (I.) so that at the end of the run it would be about as much below (I.) as it was above at the beginning. This made the value of the integral involving θ_a approximately zero and kept the maximum value of the corrections low. The water-cap with a drop of dry sperm in the fertilizing tube was then put into place, the cap filled with water and the readings begun. The starting of the experiment and the making of the observations and recording them occupied the full time of two persons. Readings of the main thermopile, θ_a, were made every 60 seconds, and of the auxiliary thermopile, θ_b, every five minutes, for a period of from two and a half to three hours. Experiments were in only a few cases continued beyond the three hour limit. When steady conditions had been reached and enough readings had been taken so that the heat production of the unfertilized eggs could be determined, the pipette was lowered until its tip was below the surface of the egg suspension, the sperm washed out and stirred into the suspension, and the pipette raised again. This operation seldom caused an irregularity of more than 0.0005° C. At the same time a sample of the same batch of eggs was fertilized in a finger bowl, and kept surrounded by running sea water. These eggs were examined from time to time. The galvanometer zero and the storage cells for the potentiometer were checked frequently. At the end of the run the average diameter of the eggs was measured, in addition to the usual data on the percentage of fertilization and development. The volume of the single egg computed from the average value of the diameter, and the total volume of eggs used was determined by centrifuging the suspension. After the centrifuging, the eggs were deformed so that their volume represented almost all of the volume measured; thus it was possible to obtain an estimate of the total number of eggs used. This method of counting was checked against a dilution method, similar to that employed for counting blood corpuscles, and it

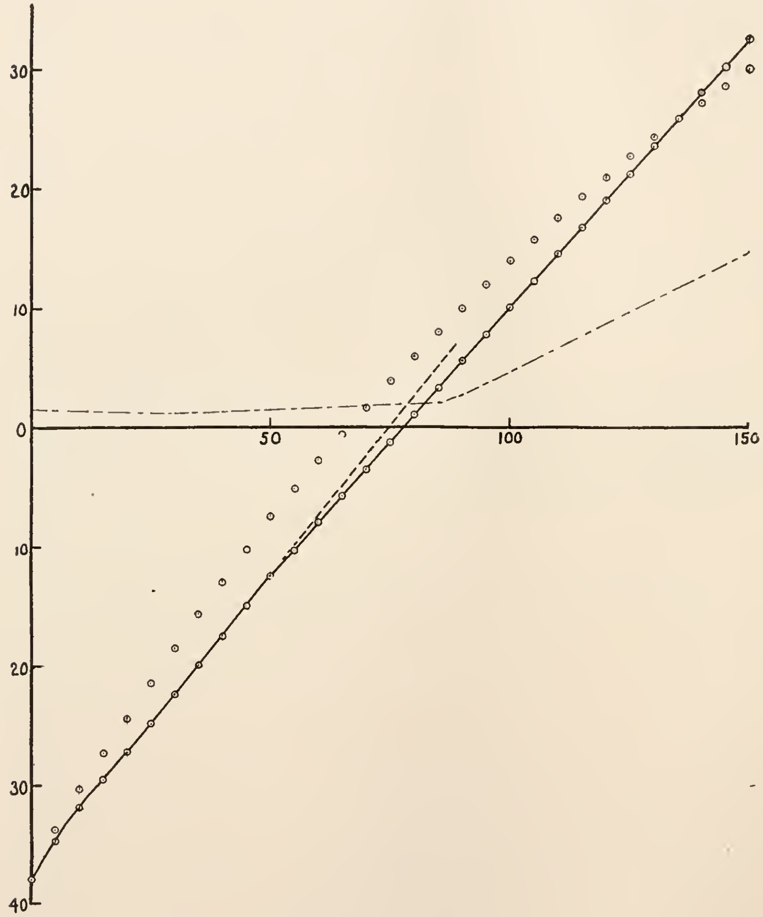

FIG. 3. Typical experimental curve. Heat production of *Arbacia* eggs. Abscissæ, time in minutes after fertilization. Ordinates, temperature differences in micro-volts. $1 \mu v = 0.001364°$ C. ⊙ θ_a observed values. — – — θ_b observed values. —⊙— corrected curve. — — — projection of corrected curve of 20 to 50 minute period beyond the 50 minute position.

was found that the agreement was very close, and that the eggs showed less variation than did the number of eggs in the fractions counted.

The course of a typical run is shown in Fig. 3. In it the average diameter of the eggs was 74 microns, and the total volume was 0.82 cc. The total number of eggs was, therefore, about 3.9 millions. About 96 per cent. of these eggs were fertilized and of those fertilized about 85 per cent. were in the eight cell stage, and the rest in a late four cell stage and going into the eight cell stage so rapidly that an accurate percentage could not be obtained, when the experiment was concluded. In Fig. 3 every fifth reading is plotted. The corrected curve was obtained by taking approximate values of the integrals of equation (3) for five minutes intervals. The greatest variation in the different runs is in the behavior during the first twenty minutes after fertilization. This is probably due to the fact that the amount of sperm could not be made proportional to the number of eggs, and that the heat production of the sperm is not negligible during this period, as will be shown later. In this

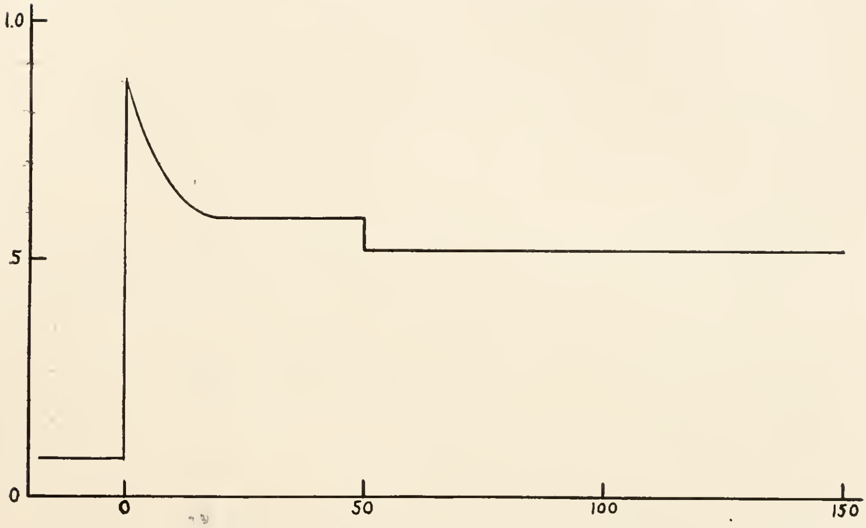

FIG. 4. Rate of heat production, *Arbacia* eggs. Abscissæ, time in minutes after fertilization. Ordinates, rate of heat production in calories per hour per million eggs.

respect the run shown in Fig. 3 shows a marked variation from the average.

The average of seven runs is shown in Fig. 4, which gives the approximate *rate* of heat production. The rate of heat production per million unfertilized eggs is about 0.08 calories per hour, while for the fertilized eggs after they have gone into the two cells stage it is about 0.52 calories per hour.[1] Both of these values are higher than those obtained by Shearer and by Myerhof with other sea urchins, but the ratio of fertilized to unfertilized eggs is the same. It should be pointed out that the results here given must be taken as indicative rather than conclusive and that further painstaking work is necessary.

One feature of the curve shown in Fig. 4 deserves special comment. It will be noted that the greatest rise in temperature, *i.e.*, the greatest period of heat production occurs immediately upon fertilization. This certainly raises again the question as to whether the process of membrane elevation depends upon an oxidative process, set up by the sperm cell when it comes into contact with the surface of the egg.

It must be mentioned here that Loeb (3) had expressed in 1906 the view that the essential feature (or possibly one of the essential features) of the process of fertilization is the increase in the rate of oxidation in the egg, and that this increase is caused by the membrane formation. Both Warburg and Loeb and Wasteneys had shown that the rate of oxidation in the sea-urchin egg is increased from 400 per cent. to 600 per cent. upon the entrance of the spermatozoön—and that membrane formation alone, induced by artificial means, has the same result. There is, therefore, a definite relation existing between membrane formation and increased rate of oxidation. From Warburg's (8) work it also seems likely that the increased oxidation occurs chiefly at the surface of the egg. The fact that the greatest heat production by the egg comes immediately after fertilization seems to us to make it plausible to say that the entrance of the spermatozoön induces a cortical oxidation process, and that this process results in the elevation of the fertilization membrane. The almost explosive character of the heat evolution seems to indicate that the oxidative process is of fundamental importance in the series of fertilization phenomena.

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[1] This involves an energy liberation of approximately 1 erg per egg per hour.

In the only run made upon sperm, ten drops of dry sperm were placed in the pipette and mixed with 50 cc. of sea water after steady conditions had been established. The heat produced can be expressed quite accurately by an equation of the form

$$H = H_0(1 - e^{-bt}),$$

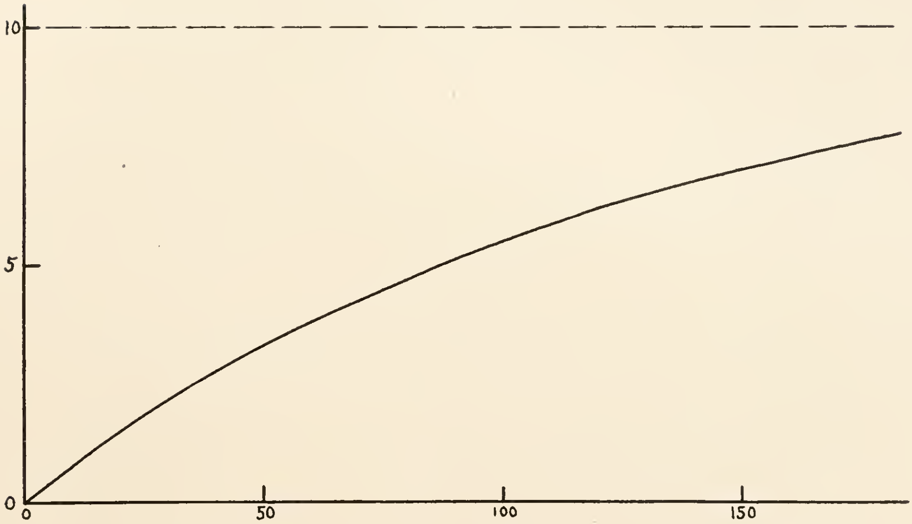

FIG. 5. Heat production of *Arbacia* sperm. Abscissæ, time in minutes after one drop of sperm is placed in 50 cc. sea water. Ordinates, temperature difference in micro-volts.

where H_0 is the total amount of heat produced—which, from the work of Cohn (1) is probably a constant for any given amount of sperm, b is probably dependent upon the pH of the water and the temperature, e is the natural base of logarithms, and t is the time after the sperm comes in contact with the water. This equation suggests very much the heat production by an exothermic reaction of the first order. In this case for ten drops of sperm,

$$H_0 = 0.79 \text{ calories,}$$
$$b = 0.008 \text{ when } t \text{ is in minutes.}$$

Figure 5 shows the temperature change when one drop of sperm (approximately the amount used) is added to 50 cc. of sea water. The curve approaches 10 micro-volts as an asymptote, but reaches 50 per cent. of that value in about 90 minutes. From this it will be seen that if there is an excess of sperm it may seriously affect results during the first twenty or thirty minutes.

As has been pointed out this method can not be used over long periods of time with the desired degree of accuracy. It also has the disadvantage of requiring large numbers of eggs, so that the longer they run, the more they tend to fall out of step, and so tend to mask any effect that may be present. This latter difficulty will remain, of course until it is possible to work with a single egg. The apparatus is being redesigned so that it will be possible to follow the heat production of both eggs and sperm under varying conditions over longer periods of time. It is planned to extend the work so as to include other forms.

SUMMARY.

The heat production of the eggs of *Arbacia punctulata* has been measured before, during, and following fertilization, through development into the eight cell stage. It has been found that the rate of heat production at the instant of fertilization is ten to twelve times that of the unfertilized egg. After fertilization the rate of heat production decreases constantly for twenty minutes, when it reaches about 65 per cent. of the value at fertilization, and remains constant until the first cleavage, at about 50 minutes after fertilization. At the first cleavage the rate drops suddenly by more than 10 per cent., and then remains constant until the eggs are in the eight cell stage, which is as far as the work has been carried. The rate of heat production of the unfertilized eggs was found to be about 0.08 calories per hour per million eggs, and that of the fertilized eggs about 0.52 calories per hour per million eggs after the one cell stage.

An experiment on *Arbacia* sperm indicates that when placed in contact with sea water, its heat production is similar to that of an exothermic chemical reaction of the first order.

The suggestion is offered that the heat evolution occurring immediately upon fertilization is the result of an oxidative process which takes place in the cortex (chiefly) of the egg and which leads to the elevation of the fertilization membrane.

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CONCERNING THE RELATIVE PHOTOTROPISM OF VESTIGIAL AND WILD TYPE *DROSOPHILA*.

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In a previous investigation upon various phases of the light and gravity reactions of *Drosophila melanogaster*, the present writer concluded among other things, that the mutant race known as vestigial is much less phototropic than is the wild fly.[1] Subsequent to this, however, W. H. Cole, using a somewhat different technique from that of the author, arrived at quite another conclusion.[2] He made his tests in a vertically placed glass tube, with the source of light above it, and under these conditions he found that vestigial flies when attempting to climb the smooth walls of the tube constantly lost their footing, and slipped back. Thus they made poorer records than did winged insects, which, when they slipped, used their wings to catch themselves. This circumstance, according to Cole, entirely accounts for any apparent difference in the phototropic response of these two types of *Drosophila*. The correctness of this interpretation he seems to have proven by lining the glass with thin rice paper which enabled the wingless animals to obtain a secure footing, under which condition they made as good records as did the flies with wings.

It is to be noted that throughout these experiments only tests with a vertically placed tube were made. This was done on the assumption that the negative response to gravity which *Drosophila* gives would be constant, and that, therefore, any additional response would be due solely to the light placed above the tube. That this was apparently true Cole showed by testing the insects when only a very dim red light was present, as well as with lights of varying intensities. Nevertheless, in view of the author's previous results it seemed to him that either this mixing of the two responses, or perhaps some other unrecognized factor, might have produced complications which would account for the discrepancy in results. It was, therefore, determined to repeat Cole's work, using so far as possible his own technique, but with the addition of tests in horizontal tubes. The method employed was as follows:

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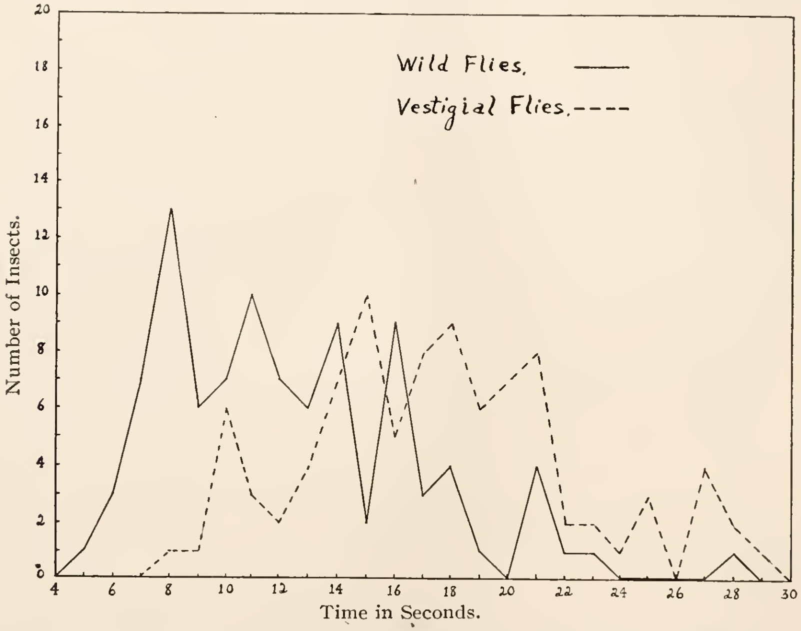
[1] McEwen, R. S., "The Reactions to Light and to Gravity in *Drosophila* and its Mutants," *Jour. Exp. Zoöl.*, 25: 49, February, 1918.

[2] Cole, W. H., "Note on the Relation Between the Photic Stimulus and the Rate of Locomotion in *Drosophila*." *Science*, LV., No. 1434, June 23, 1922.

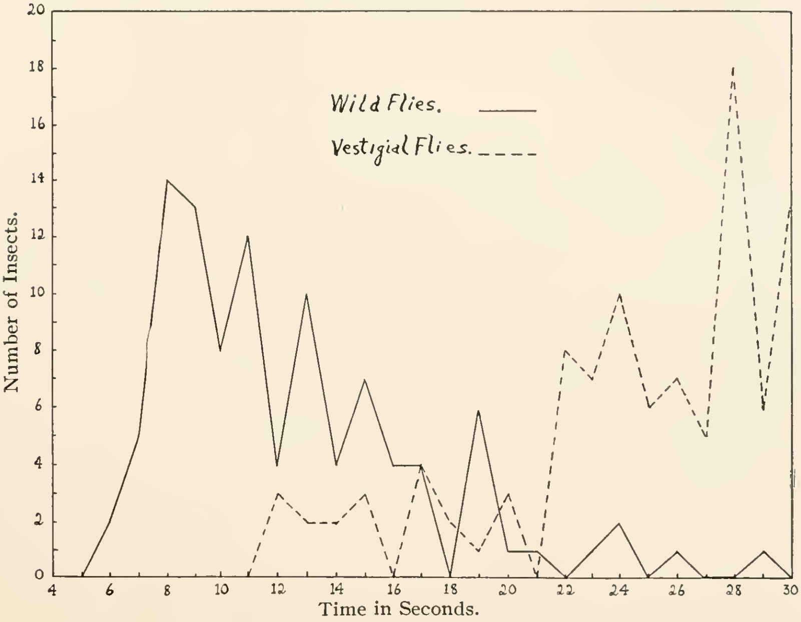
A number of wild and vestigial flies were kept in bottles containing a plentiful supply of food in which larvæ were working, until five days after hatching. On the eve of the sixth day from ten to fifteen insects of each kind and of the same sex were removed, etherized, and each animal placed in a separate vial with a small amount of food. The next morning each insect was given a preliminary test within its vial, to determine so far as possible its general activity. This was done for the vestigials by turning the glass one end up and then the other four or five times. The five vestigial flies which responded most readily by giving the negative reaction to gravity, and which showed the greatest facility in crawling up the smooth glass of the vials, were then selected for the critical tests. Five wild flies were similarly chosen, except that their reaction to light was used as a criterion of activity, and an effort was made to pick those which reacted by crawling rather than by flight. In this connection it might be thought that the use of light rather than gravity as the stimulus for the winged flies would result in the selection of particularly phototropic animals. However, since virtually all wild type *Drosophila* are decidedly phototropic, the fact that those which only crawled to the light were chosen, would mean that if anything the less phototropic ones were selected. This preliminary work was then immediately followed by the critical tests of the ten flies in a manner to be described below. A similar procedure was continued on successive days until fifty wild males and fifty vestigial males had been tested; the same was then done with fifty females of each kind, making a total of 100 insects of each type. Each day's critical tests involved the following apparatus and manipulation:

Two tubes were used, one with no lining, and the other lined with thin rice paper throughout the portion through which the fly was to crawl. Each tube measured 16 mm. in internal diameter, and the length from the end away from the light to the point where the trial terminated was 172 mm., this being identical with the distance employed by Cole. Beyond this point the tube continued for 70 mm. to the end, which was sealed with a piece of cover slip. A 75 watt gas filled lamp on a 110-volt alternating current was used as the source of illumination, and the distance from the end of the tube furthest from the light to the center of the concentrated filament of the lamp was approximately 317 mm. At the point from which the fly started, therefore, it was subject to an intensity of about 1,238 candle meters, an intensity slightly less than the maximum of 1,500 candle meters used by Cole. At the beginning of a test an insect was transferred from its vial to the testing tube by making use of its light or gravity reaction, or when necessary



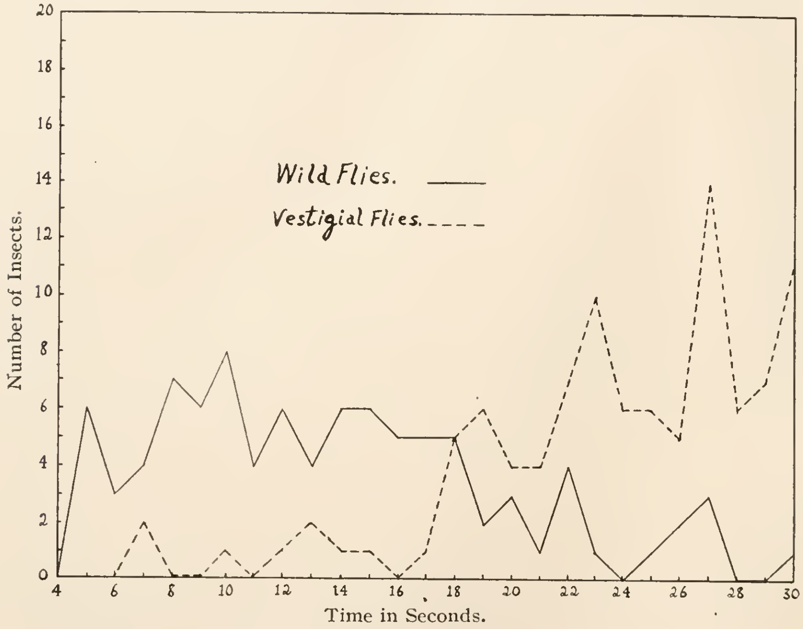
GRAPH I. The solid line shows the record, corrected for use of wings, of 95 wild flies, 45 males and 50 females, in an unlined vertical tube. The broken line, shows the record, corrected for slipping, of 92 vestigial flies, 45 males and 47 females also in an unlined vertical tube. In each case the time is plotted against the number of insects.

by shaking. The end of the tube was stopped by a cork covered with black paper, and the tube was placed in position. Ten successive trials were given in each test, the time of movement through the distance of 172 mm. being taken by a stop watch. If the passage was not accomplished within 30 seconds, a trial was considered completed, and 30 seconds was recorded as the time made. After each trial the animal was immediately returned to the starting point, if possible by its light or gravity reaction, but frequently by shaking. The latter procedure was made necessary in order that an approximately uniform period (about half a minute) might elapse between each trial. In this manner each of the five flies of a given type was tested in immediate succession, the same tube (either the one lined by paper or the one unlined) being used in the same position (either vertical or horizontal) for each insect. When the test of one group of flies was thus completed, the vials containing the insects were



GRAPH II. The solid line shows the record of 100 wild flies, 50 males and 50 females, in a lined vertical tube. The broken line shows the record of 100 vestigial flies, 50 males and 50 females, also in a lined vertical tube. In each case the time is plotted against the number of insects.

set aside in a dim light, and the test of the other group begun. In this way each of the two groups were given during the day four tests, so spaced that the time elapsing between the successive tests of any given insect was approximately two hours. Each of the four tests was of course varied as regarded either the type of tube used or its position, the four possibilities involved being:



GRAPH III. The solid line shows the record, corrected for flight, of 93 wild flies, 43 males and 50 females, in an unlined horizontal tube. The broken line shows the record of 100 vestigial flies, 50 males and 50 females, also in an unlined horizontal tube. Only three instances of slipping were noted in the case of the vestigials, and as correction for these would have made no significant difference it is not shown.

the unlined tube horizontal, the unlined tube vertical, the lined tube horizontal, and the lined tube vertical. Furthermore, for the purpose of overcoming any biased effect which might result from always testing the same type of fly in the same condition with respect to time of day and previous tests, the order of the tests was rearranged each day. Under this plan during the course of the experiments each of the two types of insects was tested under almost (though not quite) every possible combination with regard to the above factors. The variations in the arrangement of the tests thus obtained, though not entirely complete, were felt to be at least amply sufficient to avoid any significant effect from such a source of error. The results obtained by these methods are presented concretely in Table I., and Graphs I., II., and III.

TABLE I.

WILD FLIES.

Tube Horizontal.		Tube Vertical.	
Test I.,[1] Lined.	Test II., Unlined.	Test III., Lined.	Test IV., Unlined.
Male Female 12.6 12.9 Combined Average 12.8 Probable Error .287	Male Female 10.5 15.2 Combined Average 12.8 Probable Error .341	Male Female 11.7 13.9 Combined Average 12.8 Probable Error .31	Male Female 10.8 14.2 Combined Average 12.5 Probable Error .29
	Corrected for Flight Male Female 12.1 16 Combined Average 14.2 Probable Error .425		Corrected for Flight Male Female 11 14.3 Combined Average 12.7 Probable Error .308

VESTIGIAL FLIES.

Tube Horizontal.		Tube Vertical.	
Test I.,[1] Lined.	Test II., Unlined.	Test III., Lined.	Test IV., Unlined.
Male Female 24.7 26.1 Combined Average 25.4 Probable Error .25	Male Female 22.9 25.2 Combined Average 24 Probable Error .335	Male Female 24 25 Combined Average 24.6 Probable Error .325	Male Female 18.5 19.1 Combined Average 18.8 Probable Error .348
			Corrected for Slipping Male Female 17.4 18.5 Combined Average 18 Probable Error .337

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[1] These numbers are merely for convenience in designating, and do not indicate that the tests were always given in this order (see text).

As regards the mathematical procedures used in obtaining the values shown in the table and graphs the following should be said: The record of each fly in any given test was secured by taking the average of its ten trials in that test. The 100 records of all the individual insects of one type (winged or vestigial) were then in turn averaged for each of the four tests indicated. Both in making the graphs and in computing the probable errors the records of the 100 flies obtained as just described were employed, rather than the results of all their individual trials. This was the essential procedure throughout, but the details were slightly modified in certain cases for these reasons. It was realized shortly after the beginning of the experiments that in view of the question at issue it would be highly desirable to have for comparison averages which had been corrected, so far as possible, for the factor of flight in the case of the winged flies, and for slipping upon the unlined glass in the case of the vestigials. The modifications thus made necessary in the case of the winged animals in the unlined horizontal tube were as follows: Since 7 insects had been tested before observations on this point were begun, the records of these 7 had to be thrown out entirely. In the cases of the remaining 93 flies, wherever necessary new records were computed for each animal by eliminating any of its ten trials in which use of the wings in any manner had occurred. This process involved, as it turned out, 53 insects, with a total of 157 trials, which is an average of 2.9 trials per insect concerned; the highest number which any one animal thus lost was 7. The new records of these 53 flies were then averaged in with the 40 which required no change to obtain the so-called "corrected for flight" average. In the case of the winged flies in the vertical tube, correction was made in the same manner, except that in this case only the records of the first five animals had to be entirely discarded because observation on this point did not begin until after they were tested. In this instance out of the remaining 95 flies the records of 40 had to be corrected, involving 70 trials, an average of 1.75 trials per fly concerned. Again the highest loss suffered by any one animal was 7. The third correction was that for the slipping of vestigial flies in the vertical unlined tube. The same general method was employed as in the case of flight, a total of 16 animals being affected. Of these the records of 8 had to be eliminated entirely because no effort had been made to distinguish their behavior in individual trials. To the 80 trials thus counted out had to be added 11 trials distributed among the other 8 flies, the highest number lost by any one of the 8 being 3. Thus a total of 92 insects were tested, the records of 8 of them being corrected by the elimination of the trials in which slipping occurred.

It will be noted of course that the probable error in all the determinations is quite high, as the character of the curves would lead one to expect. This is due to the usual erratic behavior of *Drosophila*, which the writer has always found characteristic of this animal. Nevertheless, in view of the very marked differences between the corresponding records of wild and vestigial insects, it is felt that the results are certainly significant. Thus it may be noted that even when the probable errors are multiplied by five, the ranges so obtained do not overlap, except in a single instance. This instance is that of the range for wild flies in the horizontal unlined tube corrected for use of wings, and the range for vestigials in the vertical unlined tube corrected for slipping. Here the upper value for the range of the wild flies just equals the lower value for the range of the vestigials.

The conclusions indicated in the table and graphs may now be summarized thus: (1) The vestigials under every condition are decidedly slower than the wild type; (2) the writer's work fails entirely to confirm Cole's contention that the slipping of the vestigials is responsible for their slower records.

These conclusions come out even more clearly when the data are analyzed in more detail. Thus not only does correction for use of wings by the wild flies make no significant difference, either in the horizontal or vertical records, but correction for slipping in the case of the vestigials likewise produces essentially no effect. Curiously enough indeed, it appears both from the table and the graphs that the vestigials did much better in the vertical tests when the tube was *unlined*, while no very marked difference occurs between their records for the lined and unlined tubes in the horizontal tests. In the case of the wild flies, on the other hand, there is no significant difference between the records for the lined and unlined tubes in either the horizontal or vertical tests. In this connection it might further be stated that not only does the data based upon timing indicate that slipping is not a significant factor in retarding the vestigials, but that observation of their actual behavior leads to the same conclusion. Thus in the vast majority of cases the writer was unable to see that the vestigial insects had the slightest difficulty in crawling upon the unlined glass. Regarding the matter from yet another aspect it is instructive to note the records for corresponding groups where the only difference involved is the position of the tube. Here it appears that in the case of the wild flies in the unlined tubes the vertical position is slightly superior both as regards the arithmetical average and the mode. In the lined tubes on the other hand, only the modal value for the vertical position is superior. As regards the vestigials, the lined tubes again show only a slight superiority for the vertical records but in the case of the unlined tubes the vertical position is markedly superior, both as to average and mode, *even without the correction for slipping*. This of course is exactly opposite to what one would expect according to Cole's results and interpretation.

Besides this analysis of the data as a whole, it is also pertinent to call attention to certain facts brought out by observation of individual insects. Thus in the case of the horizontal tests of vestigial flies in the unlined tubes, where the behavior of the animals could be perfectly observed, the following feature was noteworthy. There were 14 insects which were noted on the records, not only as having no tendency to slip, but as being unusually active; *i.e.*, they crawled rapidly and constantly about. Yet the average time for these 14 cases was 23.5 seconds, virtually the same as that of the general average for the test. There were likewise 16 vestigials in this test which were recorded as "quite" active, with an average of 24 seconds, and 12 recorded as "fairly" active with an average of 27.4 seconds. Hence the mere activity of these animals does not seem to have materially affected their tendency to move toward the light. The only feasible explanation of such cases would seem to be that such flies are only weakly phototropic. This interpretation is given further emphasis by one other observation. It was noted in the horizontal tests of vestigial animals that a fly which had reached the light end of the tube would often turn immediately about and crawl quite as rapidly all the way to the dark end. Such a performance, though it would appear very favorably on the record, would obviously not denote any great degree of phototropism. This occurred, however, in at least one of the trials of each of 19 individuals. On the other hand, such action was noted in only two trials of wild type animals. In the case of vertical tests it never occurred with vestigials, but one of the two instances of wild flies took place in such a test. The reason for this single instance is not clear, and in the present state of our knowledge can only be ascribed to the erratic tendency already noted in *Drosophila*. In a similar category, perhaps, must be put the behavior of 9 vestigial insects which, though recorded as active, made relatively poor records in the vertical tests.

How the results and conclusions indicated by Cole can be reconciled with those herein presented it is rather difficult to see. In any event it is perhaps worth while summing up certain outstanding points which the two sets of experiments seem to bring out. In the first place it does not appear that the mixing of the light and gravity reactions, which was suggested as a possible source of error, has any effect upon the main question at issue. Secondly, though also apparently without bearing upon this question, is the fact that Cole's wild type flies in vertical tubes seem to have been much faster than those of the writer. With 1,500 candle meters 50 per cent. of Cole's animals averaged 6.17 seconds and with 750 candle meters 7.6 seconds. Upon the other hand, the best average obtained by the writer was 10.5 seconds, the remainder of them being from two to four seconds slower, under a stimulus of 1,238 candle meters. Such a discrepancy almost causes one to wonder whether there may not have been some fundamental difference in the character of the insects used or in the experimental conditions. Thus, for example, the tests herein recorded were conducted at an average temperature of 24.1 degrees centigrade, and it is known in a general way that the activity of *Drosophila* varies more or less directly with this factor. Unfortunately Cole gives no record of temperature. Nevertheless, so far as data on this point go, it seems improbable that his work was done at a sufficiently higher temperature to account for the difference in results. In this connection another feature of Cole's work which makes for some uncertainty is the fact that the records for his flies just cited apparently refer to only 50 per cent. of the insects tested. What the other 50 per cent. did might possibly be significant, at least as regards comparative records for the wild type. Lastly, perhaps the most pertinent point to be indicated is this. One conceivable explanation for the poor showing of Cole's vestigial animals in the unlined tube is the fact that presumably they were not previously selected for their ability to crawl on glass, as was the case to a certain extent with those used by the writer. It seems clear, however, that neither this, nor any of the other points mentioned, account at all for the fact that whereas Cole's results showed the vestigials, when given a proper footing, nearly or quite the equals of his winged flies, in the present experiments the wild type completely outclasses the vestigials under all conditions, even when correction has been made for use of wings by the former and slipping by the latter.

THYROID FEEDING AND SECONDARY SEX CHARACTERS IN RHODE ISLAND RED CHICKS.

HARRY BEAL TORREY AND BENJAMIN HORNING.

In an earlier paper [1] we called attention to the precocious appearance of the second set of rectrices in Brown Leghorn chicks whose daily ration from the fourth week after hatching had included dessicated thyroid. Reference was made also to another case of precocious development of rectrices, namely in Rhode Island Red male chicks,[2] as a result of thyroid feeding. And, correlated with the early appearance of the rectrices in the latter was a failure of the feathers on the neck to differentiate into the hackles characteristic of the control males. The acceleration of rectrices and absence of hackles of the male type combined to give to the plumage of the thyroid-fed males an aspect so strikingly female as to deceive even experienced poultry fanciers. Comb, wattles, carriage of the body and behavior remained, however, characteristically male. The accompanying tables present typical facts and the figures typical birds.

Table I. shows the effect of thyroid feeding in one experiment on the development of the first rectrices in Rhode Island Red chicks. When the latter were four weeks old, they were divided into two lots as indicated in the table. To each bird of one lot a capsule of Armour's desiccated thyroid, containing .2 per cent. I, was given daily. For the first two weeks the dose was 50 mgms.; for the next three weeks, 100 mgms.; for the next three weeks, 150 mgms. The other lot served as a control. All birds were twelve weeks old when recorded.

Figure 1 is from a photograph of the typical bird recorded as No. 1, Table I., at the age of twelve weeks. The rumpless condition is characteristic of normal Rhode Island Red males at this age. It is due to the fact that the first set of rectrices customarily

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[1] Torrey and Horning, The Effect of Thyroid Feeding on the Moulting Process and Feather Structure of the Domestic Fowl, BIOL. BULL., XLIX, 1925, 275.

[2] Torrey and Horning, Hen Feathering Induced in the Male Fowl by Feeding Thyroid, *Proc. Soc. Exper. Biol. and Med.*, XIX., 1922, 275.

TABLE I.

Control Birds.				Thyroid-fed Birds.			
No.	Sex.	Rectrices.	Type.	No.	Sex.	Rectrices.	Type.
1	Male	0	Male	11	Male	+	Female
2	Male	0	Male	12	Male	+	Female
3	Female	+	Female	13	Female	+	Female
4	Female	+	Female	14	Male	+	Female
5	Male	0	Male	15	Male	+	Female
6	Male	0	Male	16	Male	+	Female
7	Female	+	Female	17	Female	+	Female
8	Female	+	Female	18	?	+	Female
9	Female	+	Female	19	Female	+	Female
10	Male	0	Male	20	Female	+	Female

A plus sign indicates the presence of rectrices.

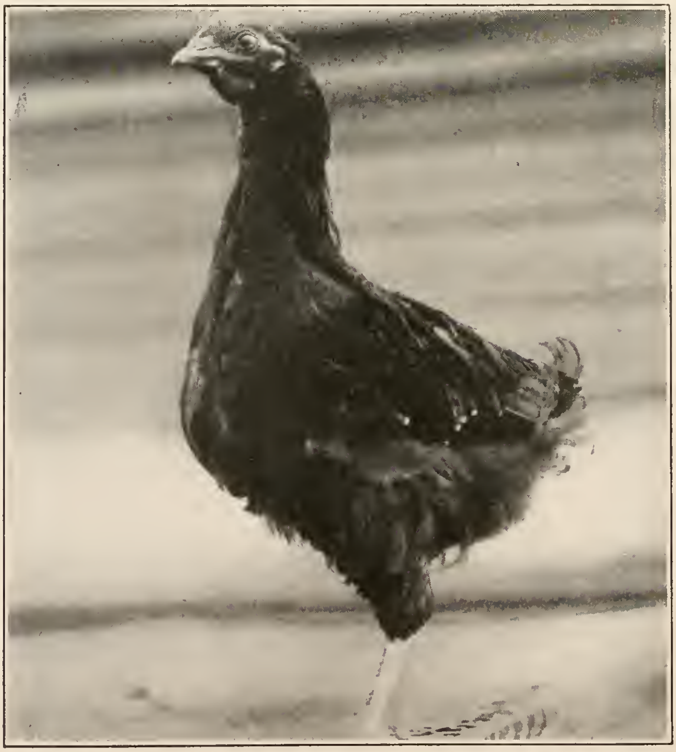

FIG. 1. Normal R. I. R. male (No. 1, Table I.) twelve weeks old. The absence of rectrices is typical at this age.

does not appear. There are exceptions to this rule, but in these cases, the rectrices are small and more or less atypical. There is some variability in the form of the first rectrices in the female, the stoutness and placement of the feathers and the shape of the feather tips being most frequently affected, less often the number. But whatever their form, the rectrices are characteristically absent in the male, present in the female.

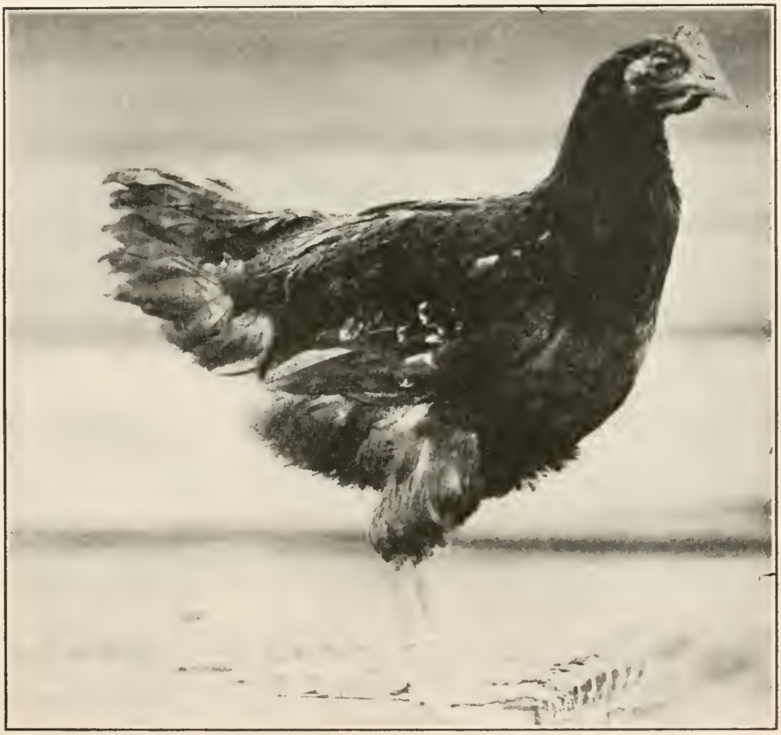

FIG. 2. R. I. R. male (No. 12 of Table I.) twelve weeks old, thyroid fed for eight weeks. Rectrices well developed.

In thyroid-fed males, they are characteristically present also, appearing about the time they appear in normal females. Fig. 2 is from a photograph of No. 12, Table I., twelve weeks old. Fig. 3 is another thyroid-fed bird, aged twelve weeks, being No. 15, Table I. In each case the head is typically male, the feathering typically female. Comparison of Figs. 2 and 3 with Figs. 4 and 5 will emphasize these facts. Fig. 4 is from a photograph of No. 4 Table I, a typical control female twelve weeks old. Fig. 5 is from a photograph of No. 13, Table I, a typical thyroid-fed female of the same age.

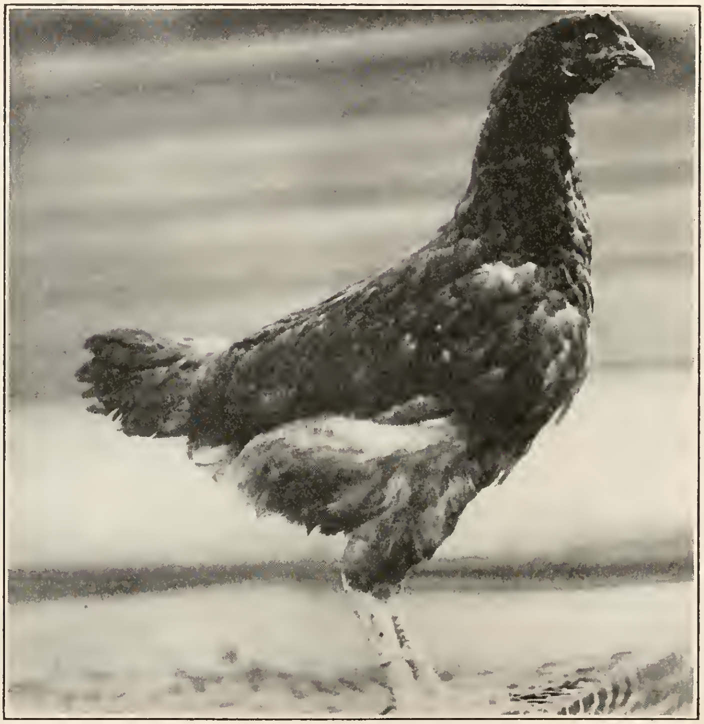

FIG. 3. R. I. R. male (No. 15 of Table I.) twelve weeks old, thyroid fed for eight weeks. Rectrices well developed.

There was no essential difference between the last two birds at the time the photographs were taken. The conclusion, however, that thyroid feeding does not affect the female plumage at this age must not be hastily drawn. When thyroid-fed chicks are compared with controls at an earlier age, evidence is obtained to show that thyroid feeding accelerates the development of both rectrices and contour feathers in females as well as males.

In Table II. such evidence is summarized. The chicks were 51 days old. Eight days after hatching, thyroid feeding had been begun with 15 mgms. as the initial daily ration, in capsule as before. This had been increased from time to time, the chicks being fed as much thyroid as they could stand without displaying obvious signs of weakness or distress.

TABLE II.

Control Birds.				Thyroid-fed Birds.			
No.	Sex.	Rectrices.	Contours.	No.	Sex.	Rectrices.	Contours.
65	Female	1 cm.	Not well out	81	Female	?	Not well out
66	Female	0	" " "	82	Female	7.5 cm.	Well out
67	Female	0	" " "	83	Female	2.5 cm.	" "
68	Female	0	" " "	84	Female	1 cm.	" "
69	Female	0	" " "	85	Female	5 cm.	" "
70	Female	0	" " "	86	Female	2.5 cm.	" "
71	Female	0	" " "	87	Female	2.5 cm.	" "
72	Female	0	" " "	88	Female	0	" "
73	Male	0	" " "	89	Male	0	Not well out
74	Male	0	" " "	90	Male	2.5 cm.	Fairly well out
75	Male	0	" " "	91	Male	1 cm.	Well out
76	Male	0	" " "	92	Male	1 cm.	" "
77	Male	0	" " "	93	Male	4 cm.	" "
78	Male	0	" " "	94	Male	2.5 cm.	Not well out
79	Male	0	" " "	95	Male	4.5 cm.	Well out
80	Male	0	" " "	96	Male	4.5 cm.	" "
				97	Male	0	Not well out

It will be seen on inspection of Table II, that there is considerable variation in the length of the rectrices and the condition of the contour feathers in thyroid-fed birds of both sexes. There are differences, not here recorded, in size and weight also, that are not, however, strictly correlated with differences in the feathering.

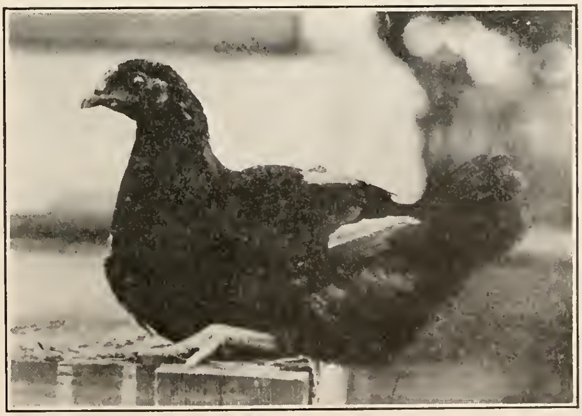

FIG. 4. Normal R. I. R. female (No. 4, Table I.) twelve weeks old.

We are aware that such variability is a source of difficulty in the interpretation of results. It is greatest in the first few weeks after hatching, when it is attributable partly to irregularities in nutrition and to differences of vigor referable to other causes. These tend to disappear with age. But the constitutional variability of the Rhode Island Red breed, which we have learned to recognize in the course of our work, remains as a source of error. All of our experiments have been checked in other breeds, notably in Leghorns (Torrey and Horning, 1925) and Campines, where the forcing effect of thyroid feeding on the development of the plumage is clear and definite. In the latter breeds, however, the first rectrices normally develop simultaneously in both sexes.

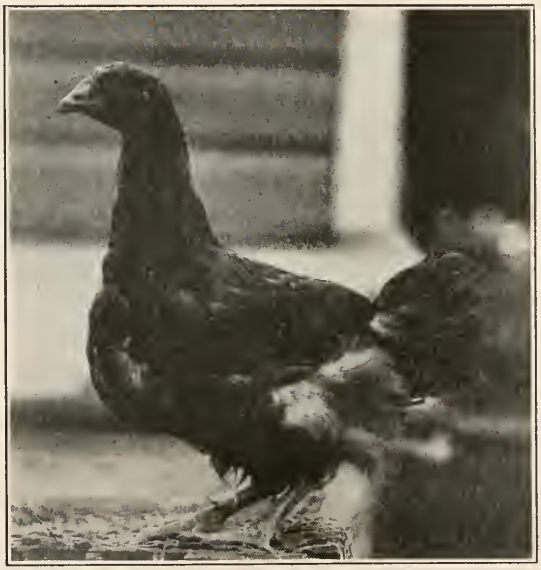

FIG. 5. R. I. R. female (No. 13, Table I.) twelve weeks old; thyroid fed for eight weeks.

The variability of Rhode Island Reds led us formerly into a conclusion that proved to be erroneous. In the first series of experiments reported, our five thyroid-fed capons failed to develop rectrices like unaltered thyroid-fed males. Later it was discovered that this result was exceptional. The gonad had, in the male, no bearing on the result.

This misconception led us to suspect that the effect of thyroid feeding on the feathering of the male might be indirect, through an augmentation of the so-called luteal tissue, to whose presence in the testes of Campines and Sebright bantams has been imputed the hen-feathered condition of the males of these breeds. No support for this suspicion was obtained from histological preparations, however, and the observation of the thyroid effect in capons completely disposed of the possibility of it. But that the thyroid effect is not independent of the ovary in certain respects is a fact that will be developed in another connection.

The intensity of hen feathering in males of hen-feathered breeds differs with the breed. For instance, the male silver Sebright bantam is an absolute replica of the female, in plumage. The male silver Campine, however, though resembling the female closely, differs from it not only in the possession of sickle feathers but in the structure of the hackles, which are laced in the typical male fashion.

Now the plumage of thyroid-fed Rhode Island Red males, up to the age of 12 weeks, is indistinguishable from that of females. Later on, the males in our experiments developed plumage that is perhaps best described by saying that it was prevailingly male with certain modifications characteristic of the female type.

In place of typical male hackles (Fig. 6, *a*, *b*) with broad marginal lacing of naked barbs, and with sharply pointed tips, thyroid feeding produced hackles with broader and more rounded or truncated tips, and narrower lacing, especially toward the tips, but, more or less irregularly, elsewhere also (*c*, *d*). Saddle feathers (Fig. 7, *a*, *c*) were similarly affected, the ends being broader and more rounded, and the laced margin furnished with an irregular inner border owing to the development of barbules at points where the barbs are normally naked (Fig. 7, *b*) or in exceptional cases, to the absence of barbules in normal situations. We shall have occasion to discuss these irregularities elsewhere.

The characteristically broad distal lacing of shoulder feathers in normal males (Fig. 8, *a*) was still more strikingly reduced in thyroid-fed males (*b*), often, in fact, completely obliterated (*c*), giving the feathers an undeniably female aspect, as can readily be seen by comparing (*c*) with (*d*), the latter being a feather from the shoulder of a normal female.

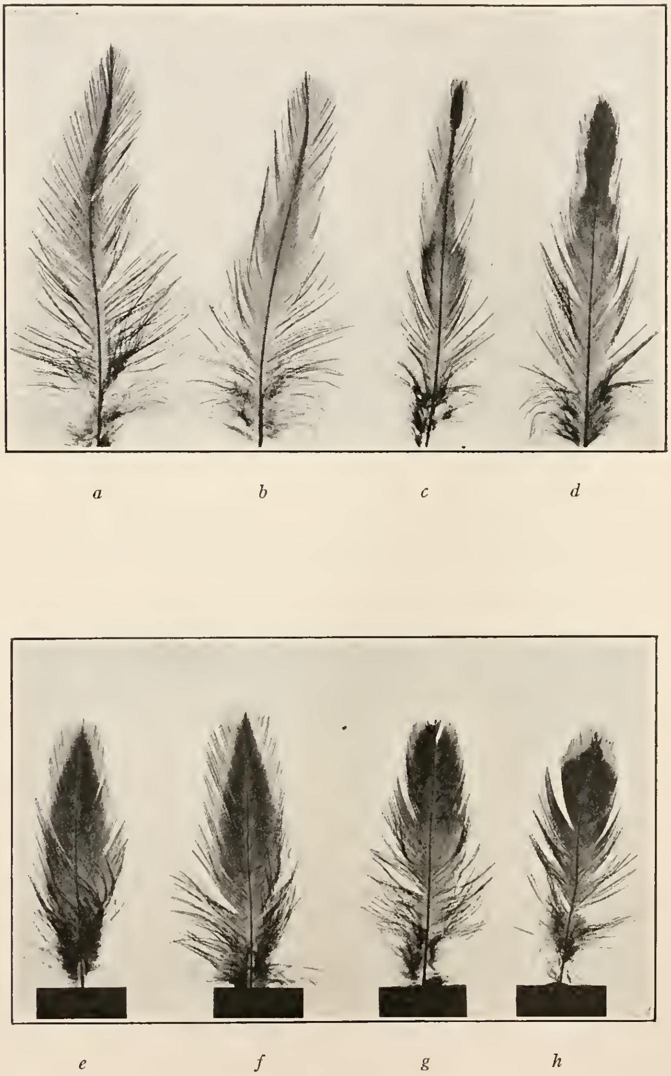

FIG. 6. Hackles from R. I. R. birds about seven months old: *a*, *b*, from a normal male; *c*, *d*, from a thyroid-fed male; *e*, *f*, from a normal female; *g*, *h*, from a thyroid-fed female.

To a less but still striking degree, the hackles of the thyroid-fed males tended to assume the aspect of female hackles. Compare, with this in mind, Fig. 6, *a*, *b*, with Fig. 6, *e*, *f*, *g*, *h*. And note also that, in this series of feathers, the hackles of the female, which are here exceptionally male in structure, are modified in the same direction.

To a still less degree than the hackles do the saddle feathers of thyroid-fed males approximate the saddles of normal females: yet here too the changed shape of the feather and the added barbules and consequent diminished lacing suggest the female type.

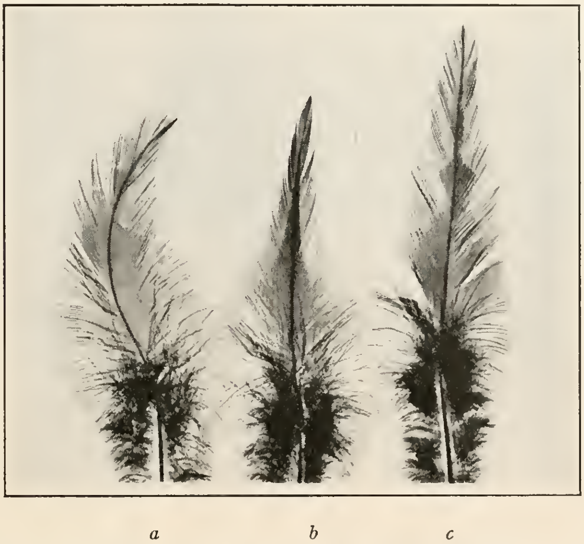

FIG. 7. Saddle feathers from R. I. R. birds about seven months old: *b*, from a normal male; *a*, *c*, from a thyroid-fed male.

By way of summary it may be said that when male Rhode Island Red chicks were fed desiccated thyroid in daily doses increasing with their weight, two general effects were observed:

1. Their plumage appeared precociously but differentiated later than usual, so that at the age of 12 weeks they were feathered like females. It was to this effect that our earlier observations referred.

2. Their adult plumage, though prevailingly male in type, exhibited characteristics of form and structure, especially in the

hackle, saddle, back and shoulder feathers, that are found ordinarily only in the corresponding feathers of females.[1]

And it may further be said that when the lacing characteristic of the male hackle appears also in the female that the effect of thyroid is to modify it also, as in the male, by the addition of barbules.

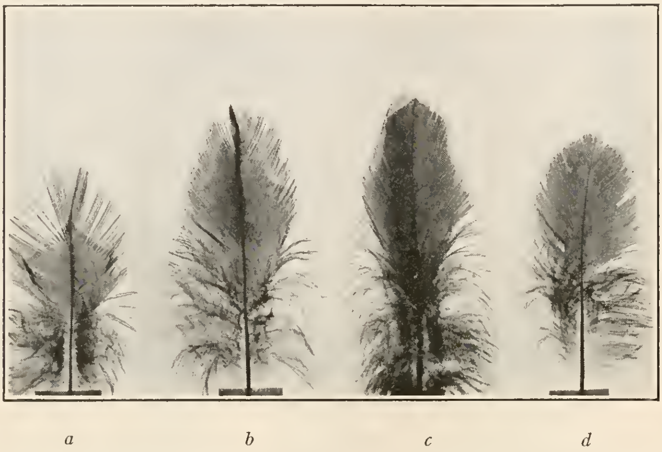

Fig. 8. Shoulder feathers from R. I. R. birds about seven months old: *a*, from a normal male; *b*, *c*, from a thyroid-fed male; *d*, from a normal female.

Thyroid feeding, then, tends to produce hen feathering in the Rhode Island Red male, and, to the extent noted in the last paragraph, in the female also. Toward this result there is no coöperation of the gonad. That the ovary does influence the action of thyroid with reference to certain characters not, however, correlated with sex, will be shown in another paper.

Zoölogical Laboratory,
University of Oregon,
April 7, 1925.

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[1] Crew and Huxley (*Veter. Jour.*, LXXIX., No. 10) appear to have seen neither of these effects in their thyroid-fed fowls. The first naturally escaped them, for their birds were too old at the beginning of the experiment to develop it. That the second effect also escaped them may perhaps be attributed to their material and methods. Their 12 birds, of which 6 were males, were F_1 hybrids from a cross between a Rhode Island Red ♂ and a Light Sussex ♀, and inherited the black hackles and white ground color from the mother. An amount of thyroid equal to 2 grains per bird was mixed every day into the common ration of wet mash. This dosage was smaller than ours, was not increased as the weight of the birds increased, and was so administered as to leave in doubt just how much each male obtained

STUDIES ON THE SECONDARY SEXUAL CHARACTERS OF CRAYFISHES. IV. MALES WITH TWO SETS OF SUPERNUMERARY MALE CHARACTERS.

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In study number three of this series, males of *Cambarus propinquus* and of *Cambarus virilis* which bore supernumerary male characters were described. These supernumerary characters were in the form of copulatory hooks ordinarily found only upon the third walking legs but in these cases present also upon the second or the fourth walking legs. In *C. virilis* the extra hooks were confined to the second walking legs. In *C. propinquus* the additional hooks were found upon either the second or the fourth walking legs. It was predicted that in *C. propinquus*, since either the second or the fourth walking legs might function in carrying the extra hooks, specimens might be found in which copulatory hooks would be present upon both the second and the fourth walking legs.

During the summer of 1924 large collections were made from practically all the streams and some of the lakes of southeastern Wisconsin and in one stream three males of *C. propinquus* were found which bore supernumerary copulatory hooks upon one or both of the second and fourth walking legs.

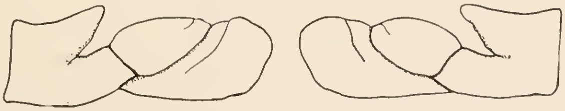

FIG. 1. Diagram illustrating the arrangement of the copulatory hooks upon the third walking legs of a normal male specimen.

DESCRIPTION OF SPECIMENS.

Specimen number one (Fig. 2) is a male of the first form and is 52 mm. long. Normal copulatory hooks are present upon the third walking legs. Hooks a little smaller than those upon the third walking legs are present upon the right second and the right fourth walking legs. The left second and the left fourth legs possess no hooks.

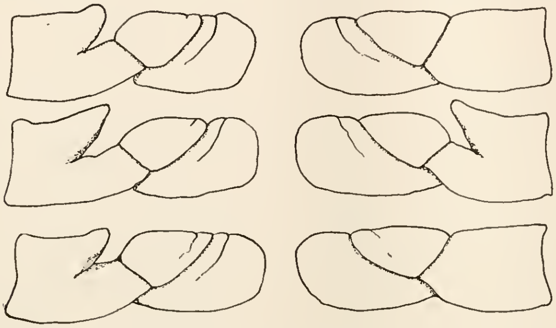

FIG. 2. Diagram illustrating the copulatory hooks upon the second, third and fourth legs as they occur in specimen No. 1.

Specimen number two (Fig. 3), also a first form male, is 43 mm. long and in addition to the usual hooks upon the third legs there are hooks also upon both of the second walking legs and upon the left fourth leg. The supernumerary hooks are again distinct but smaller than the normal ones upon the third walking legs.

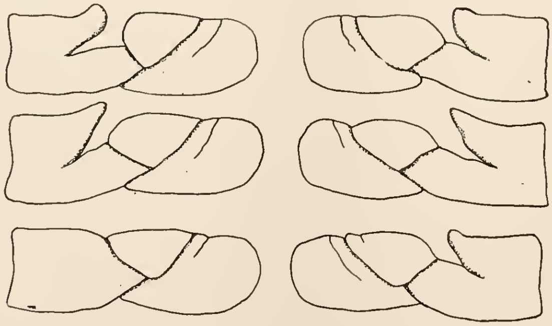

FIG. 3. Diagram illustrating the arrangement of the copulatory hooks upon the second, third and fourth walking legs in specimen No. 2.

Specimen number three (Fig. 4) is 47 mm. long and has copulatory hooks upon both of the second, third and fourth walking legs. The hooks upon the second and upon the fourth legs are slightly smaller than the normal hooks.

FREQUENCY OF OCCURRENCE AND DISTRIBUTION.

The three specimens described here were the only ones taken among approximately 3,600 males of *Cambarus propinquus*. The condition may, therefore, be considered rare.

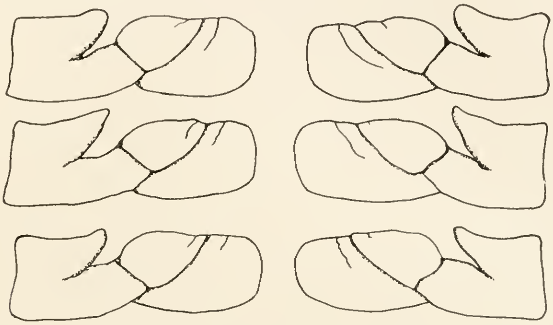

FIG. 4. Diagram illustrating three full pairs of copulatory hooks as they occur upon the second, third and fourth walking legs of specimen No. 3.

All three specimens were taken in a single section of one stream (Pike River near Racine, Wisconsin) and constituted about three per cent. of the males of the entire catch. Thirty-six other streams and rivers examined contained no aberrant specimens of this type.

Male specimens with one pair of supernumerary hooks are sometimes fairly abundant, in some localities representing as high a proportion as seven or eight per cent. of all the males. In Pike River, however, where the three specimens with two supernumerary pairs of hooks were taken, fifteen per cent of the males had one supernumerary pair of hooks, some of them bearing the extra hooks upon the second and others upon the fourth walking legs.

DISCUSSION.

Some direct and considerable indirect evidence has been accumulated indicating that in female crayfishes with male secondary sexual characters the condition is inherited and should be considered a germinal mutation. It is significant in this connection that all three specimens of the males with two pairs of supernumerary hooks should come from the same locality. It is also suggestive that in this locality there should also occur by far the largest proportion of males with one supernumerary pair of hooks. If it be assumed that the condition represented by the possession of supernumerary hooks upon the second legs is independent of the condition in which supernumerary hooks occur upon the fourth legs and that both conditions are inherited then it would follow that a combination of the two characters would be most reasonably expected in that locality where both were most abundant. Or, if it be assumed that there is present a cumulative, heritable factor which tends to produce one supernumerary character in the simplex state and added supernumerary characters when doubled then the locality in which doubling would be expected would be that locality in which the highest proportion of single supernumerary characters appeared.

This evidence for the inheritance of the characters described is not convincing in itself but it is important in that it supports the interpretation tentatively advanced for the occurrence of like aberrant structures found in females.

STUDIES ON THE SECONDARY SEXUAL CHARACTERS OF CRAYFISHES. V. MALES WITH FEMALE CHARACTERS.

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A number of crayfishes belonging to the genus *Cambarus* in which secondary sexual characters of both sexes were found upon the same individual have been described by Faxon, Hay, Ortman and the writer. All of these specimens with the exception of one described by Hay were females. In some cases there was a full complement of female secondary sexual characters with the addition of one or more male characters and in other cases a female character was lacking while one or more male characters were present. The one exceptional specimen described by Hay was hermaphroditic and possessed both male and female primary and secondary sexual structures.

Male crayfishes in the genus *Cambarus* bearing sexual characters of females have not been noted before and it is the purpose of this paper to describe two specimens which appeared in recent collections made in southeastern Wisconsin. Both specimens were collected from tributaries of the Rock River near Beloit, Wisconsin, the first in July, 1923, and the second in July, 1924.

One is a specimen of *Cambarus virilis*, 65 mm. long and is a second form male normal in every respect except that an oviducal opening exists upon the left third walking leg. An examination of the internal organs shows a normal spermary and normal spermatic ducts. There is no tube of any kind joined to the oviducal opening at the base of the third walking leg. The second specimen is one of *C. propinquus*, 46 mm. long, and is identical in its peculiarities with the first specimen described. It is a normal second form male with all the normal internal and external structures of the male but bearing in addition an oviducal opening at the base of the third walking leg. As in the previously described specimen there is no duct attached to the aberrant oviducal opening.

DISCUSSION.

Since all the specimens taken previously which bore secondary sexual characters of both sexes in the same individual have been females or hermaphrodites the impression might easily arise that some mechanism exists which is capable of producing male secondary sexual characters in females but that either the same or some other mechanism prohibits the reciprocal production of female characters in males. Indeed, the following statement with this implication is made by Hay in a discussion upon "Hermaphroditism in Crayfishes."[1] "It would appear, therefore, that in the genus *Cambarus* at least hermaphroditic individuals are females which, owing to some ambiguity of the formative cells in the embryo, have developed to a greater or less degree characters of the opposite sex." The finding of only two males having each a single female character furnishes extremely meager data upon which to generalize but it seems important to demonstrate that crayfishes having secondary sexual characters of both sexes may sometimes be males and that no influence exists in the male which completely inhibits the development of female characters.

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[1] Smithsonian Miscellaneous Collections. Sept. 8, 1905.

PHYSIOLOGICAL STUDIES ON HIBERNATION IN THE POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* SAY.[1]

DAVID E. FINK.

(From the Bureau of Entomology, United States Department of Agriculture,
Washington, and the Zoölogical Laboratory, University
of Pennsylvania, Philadelphia.)

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INTRODUCTION.

An inquiry into the nature and cause of the phenomenon of hibernation has for many years engaged the attention of investigators. The literature on the physiological study of hibernating mammals covers a wide range of research, the most significant of which appears to be into the effects of temperature, food and gaseous exchange. The experimental evidence brought forward by Dubois (10), Pembrey (17), Valentin (25), Weinland and Riehl (29) and others, indicates that hibernating animals placed in the presence of an abundance of food and warmth remain impassive. This has led to the conclusion that hibernation is some inherent, deep seated phenomenon. Recent studies by Carlier (8), Rasmussen (18), Sheldon (22) and others, upon the nature and function of the so-called hibernating gland, show it widely present in mammals.

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[1] A thesis in zoölogy presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

The literature on physiological studies of hibernating insects is not so extensive. Baumberger (1), Bodine (3), Breitenbecher (6), Roubaud (19), Sanderson (20), Tower (24) and others, however, have investigated problems of temperature, moisture, and gaseous exchange as they relate to insects. According to Baumberger (2), hibernation has resulted from the repeated effect of winter upon the species and the rhythmical phenomenon has been determined by the habits of the insect. Roubaud (19) recently advanced the view that a lowered metabolic activity permits a progressive physiological purification. According to this author, two factors, *anhydrobiosis*, and *athermobiosis* (dehydration and absence of heat), in themselves inhibitory, become the unexpected factors of reactivation by favoring the processes of excretion during dormancy.

Most investigators whether dealing with hibernation in vertebrates or invertebrates agree that the influence of temperature in the process is subordinated, and that hibernation is preceded usually by a period of extensive feeding and a consequent reduced metabolic activity. It is also well known that food is an important factor in influencing the habits of animals. The manifold instances of food storage indicate a varying adaptability, the most significant being the storage within the body of the animal of reserve nutrients in the form of fat.

With insects the phenomenon of hibernation is unique, because it may occur in any stage of the life cycle of different insects, from the egg to the adult. It seemed advisable, therefore, to investigate the problem of nutrient storage in insects and to determine the specific mode of its utilization during the progress of hibernation.

This contribution presents the results obtained from physiological studies on the potato beetle, *Leptinotarsa decemlineata* Say, before, during, and after hibernation.

The author desires to express his appreciation to Prof. C. E. McClung, and other members of the Zoölogical Department of the University of Pennsylvania, for generous aid and suggestions. To Doctor J. H. Bodine he is deeply indebted for helpful criticism during the progress of the work and for advising investigation of this problem.

Material and Methods.

The adult potato beetle, *Leptinotarsa decemlineata* Say, used exclusively in these investigations, was reared from eggs deposited by overwintering adults.

The methods adopted for different lines of investigation varied. The type of cage for determining the effect of various food plants consisted of ordinary six-inch flower pots filled with soil, in the center of which was sunk a 5 x 1 inch glass vial containing water and fresh food plant. The covering to this cage was made of mosquito wire in the form of a circular cage of a diameter to fit snugly within the inner upper rim of the flower pot, and varying in height from 12 to 18 inches. These cages were set in troughs of soil in an outdoor breeding house. A more elaborate cage consisted of a wooden framework (20 x 18 x 14 inches), constructed of inch pine, enclosed with mosquito wire and set on

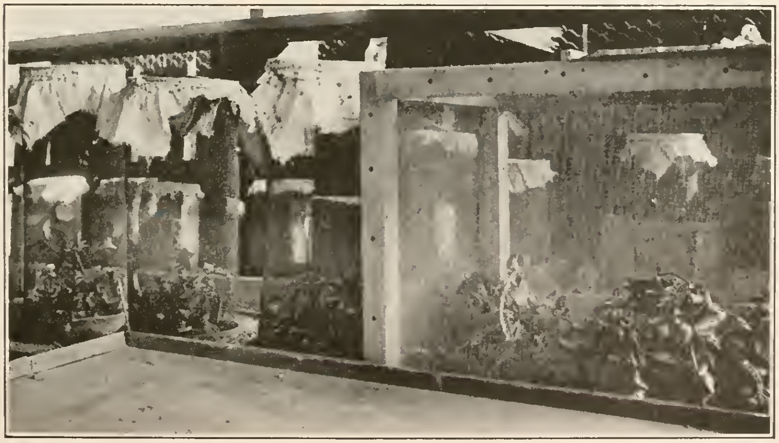

Fig. 1. Types of cages used in the food experiments. This figure shows two types: (1) circular cages composed of flower pots sunk in the soil with mosquito wire tops; (2) large oblong cages constructed of inch pine, covered with mosquito wire and set on the surface of the soil. Foliage was kept fresh in the cages by inserting them in vials of water.

the surface of the soil in the breeding house or in the field (Fig. 1). In all cages it was impossible for beetles to escape or for others to find entrance from the exterior. Access for the worker was by a convenient opening on the top or side of the cage.

The water content was obtained by first weighing and slitting the animals and afterwards placing them in an oven at a temperature between 90–95° C., until a constant weight was obtained. The percentage of water was computed on the basis of dry weight.

Although the extraction of fat from the body of an organism with solvents cannot be achieved quantitatively without change in the nature of the compounds in which the fatty acids are present, this was not considered an objection in these experiments as the result desired was the total fat content in whatever form it might occur. The tissues, however, had to be dried and powdered. To prevent partial oxidation of the more unstable unsaturated acids, the drying took place at room temperature, or in an oven at a temperature not exceeding 30° C. The extracting solvent used extensively was a mixture of equal proportions of ether and alcohol, which was warmed and renewed several times by decantation. In addition, the method described by Voltz (28), and modified to suit the material under investigation was also used. Calculations were made in percentage based on dry weight of the organism.

Metabolism determinations (respiratory exchange) were obtained by methods essentially similar to those described by the author (12) in a previous publication. Other methods used will be described in the text under appropriate headings.

Experiments and Discussion.

Food Experiments.

The effect of different food plants on potato beetles of the first generation emerging from pupation was studied. Altogether, during June, 1923, and 1924, about 800 beetles were divided into four main groups and fed on various food plants as follows:

Group 1. The foliage of potato, tomato, eggplant and peppers.
Group 2. The tubers or fruit of the above plants.
Group 3. The foliage of potato for six days, followed by sliced

non-solanaceous foods like beets, carrots, squash and cucumbers.

Group 4. Non-solanaceous foods exclusively.

Number of Days Feeding Before[1] Hibernation.

The number of days feeding on different food plants before hibernation took place is shown in Tables I.–IV., and in Fig. 2. Group 1, fed on foliage required an average of 16 days, whereas group 2, fed on tubers and fruit required an average of 13 days. Evidently, to accumulate sufficient nutrients necessitated a more extensive feeding on foliage than on tuber or fruit. The experiment with group 3, fed on potato foliage for six days followed by a period of feeding on sliced non-solanaceous foods, resulted in, first, an increase in the number of feeding days essential for nutrient storage to an average of 23.6 days; second, a mortality of 23 per cent.; third, unsuccessful hibernation of 32 per cent. (which died during the process).

Table I.

Number of Days for Beetles Fed on Foliage to Enter Hibernation.

Potato.		Tomato.		Eggplant.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
75	14	100	15	80	11
25	21			20	20
Average	17.5		15		15.5
Cages Receiving No Water.					
80	11	10	11	90	18
10	13	80	13	10	20
10	Died	10	Died		
Average	12		12		19

The general effect with group 4, fed on sliced non-solanaceous foods exclusively and those members of group 1, which were fed on pepper plants or its fruit, was one of starvation. Some died after 7 days; others lived for 41 days but were unable to hiber-

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[1] Potato beetles hibernating in July or November exhibit the same physiological reactions.

Table II.

Number of Days for Beetles Fed on Tubers and Fruit to Enter Hibernation.

Potato.		Tomato.		Eggplant.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
80	13	10	11	50	11
20	15	80	13	50	18
		10	Died		
Average	14		12		14.5
Cages Receiving No Water.					
80	11	10	11	10	11
20	13	90	18	90	13
Average	12		14.5		12

Table III.

Number of Days for Beetles Fed on Foliage Followed by Sliced Tubers and Non-solanaceous Foods to Enter a State of Hibernation.

Potato.		Squash.		Cucumbers.		Beets.		Carrots.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
75	15	80	25	40	15	40	13	40	15
25	25	20	35	50	21	50	38	10	38
				10	Died	10	Died	50	Died
Average	20		30		18		25.5		25

Table IV.

Beetles Fed on Non-solanaceous Foods Showing Number of Days before Death.

Squash.		Cucumbers.		Beets.		Carrots.		No Food.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
40	37	40	15	60	37	40	15	100	11
60	45	40	26	40	39	60	38		
		20	34						
Average	41		25		38		24		

nate. In control cages where nothing was fed, the beetles died after 11 days.

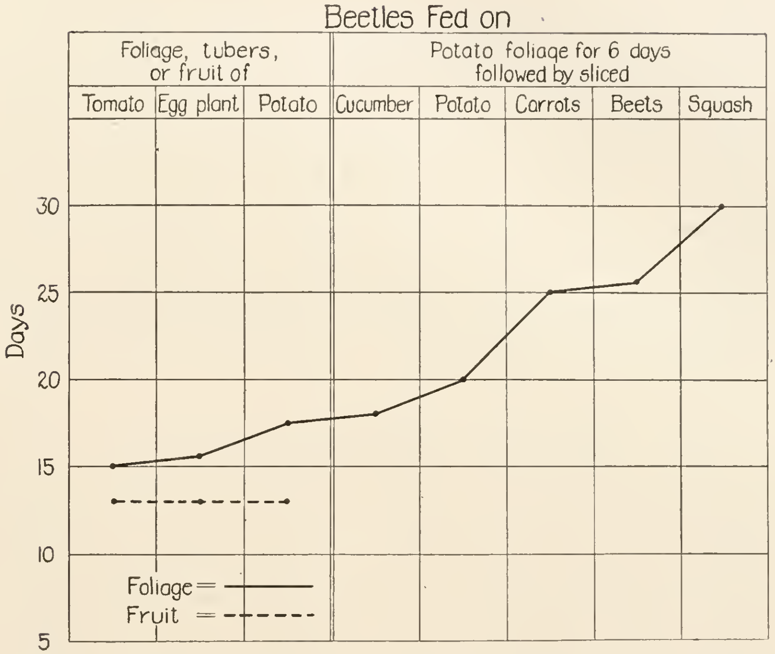

FIG. 2. Represents the number of feeding days required on different foods before the beetles entered a state of hibernation. Days are represented as ordinates, different foods as abscissa. The solid line curve shows the average number of days feeding on different foods before hibernation. The dotted line shows the average number of days feeding on tuber or fruit before hibernation.

In duplicate series of experiments conducted at the same time, certain cages received water to replace usual evaporation from the soil, others received no water. The addition of water to cages containing normal food plants decreased the feeding period from an average of 14.5 to 13.4 days. In control cages receiving only water the beetles died after 16 days. In cages containing non-solanaceous foods, the addition of water seemed to prolong the life of the beetles without inducing hibernation.

Breeding Activities.

It is perhaps essential to emphasize that potato beetles of the first generation used exclusively in these investigations indicated no breeding activities before dormancy set in. The beetles hibernated during July and August and remained in that state until the following season. Other experiments conducted by confining first generation beetles with normal food plants in field cages, produced similar results. During fall and winter these hibernating beetles were frequently removed from the soil in the cages, and sections made of the testes revealed mature sperm in abundance. Dissections of the females yielded immature ova. These results are contrary to those obtained by Tower (24), who states, "The first brood on emergence feeds for a few days and then deposits eggs for a second generation. The second generation does not develop the germ cells nor show any reproductive activity until after it has passed through a period of hibernation or æstivation."

Metabolism During Feeding Experiments.

During the progress of feeding a veritable storing of food in the form of fat takes place in the adipose tissues of the animal (Fig. 4). It has been pointed out above that a numerical difference in days exists, for nutrient storage when different foods are fed. From metabolism determinations it seems possible to correlate the results procured during the feeding experiments with the oxygen consumption. In Fig. 3, the oxygen intake per gram organism in cubic millimeters per hour is shown. From an examination of this figure it is evident that a reduced oxygen consumption takes place when tuber or fruit material, as compared to foliage, is fed. Obviously then the process of converting tuber material required less oxygen. The analysis of food plants, given in Table V., shows the amounts of carbohydrate and fat

TABLE V.

SHOWING ANALYSIS OF FOOD PLANTS IN PERCENTAGE.

Food Plant.	Water.	Protein.	Fat.	Carbohydrate.
Beets	87.5	1.2	0.1	9.4
Carrots	88.2	0.7	0.4	8.9
Cucumbers	95.4	0.6	0.2	3.0
Eggplant	92.9	0.9	0.3	4.9
Potato	78.3	1.7	0.1	17.7
Squash	88.3	1.1	0.5	8.6
Tomato	94.3	0.7	0.4	3.8

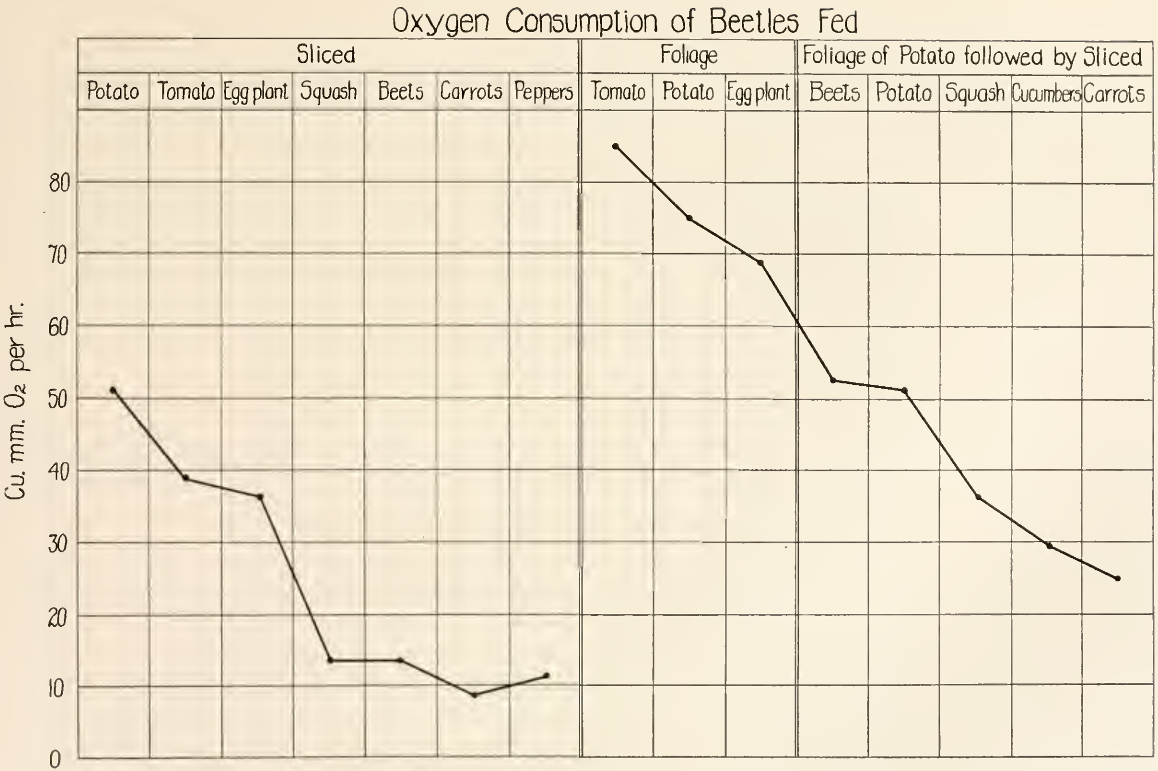

FIG. 3. Ordinates shows the oxygen intake in cubic millimeters per hour per gram body weight of beetles fed on different foods represented as abscissas. By comparison it is noted that distinctive differences in oxygen consumed are evident when potato beetles are fed tubers and fruit as compared to foliage of the same plants. This figure also shows the low oxygen intake when fed non-solanaceous foods.

present in non-solanaceous foods as compared to potato tuber and foliage. It is known that a diet rich in carbohydrates, as for example, turnips or potatoes, increases the proportion of lower fatty acids, while a diet rich in proteins diminishes this proportion. If the percentage of the lower fatty acids increases with the amount and kind of food, this fact may explain the results shown in the above experiments, since tuber material is rich in carbohydrates.

It was previously pointed out that members of group 4, fed non-solanaceous food plants and those beetles fed on peppers, in general, gradually starved. The respiratory metabolism of such animals manifests an extremely reduced oxygen consumption and (in other experiments to be discussed later) a decreased CO_2 output. In control experiments, where nothing was fed, the oxygen intake similarly was exceedingly diminished.

Preparation for Hibernation.

Of significance are the variations in weight recorded during the preparation for hibernation. First generation beetles as they emerged from pupation and before feeding averaged 0.1260 grams in weight. After feeding on foliage of the potato plant for ten days, the weight averaged 0.2627 grams. A state of quiescence or *prehibernation* [2] followed, varying from three to five days and was accompanied by a reduction of the water content and elimination of the waste material from the digestive tract, giving an average weight of 0.1879 grams. The increase in weight accounts, in part, for the accumulation of reserve nutrient material in the adipose tissues, and may, in addition, indicate growth of somatic and germinal cells. Furthermore, during *prehibernation* a gradual lowering of all vital activities takes place, the beetles afterwards entering the ground to hibernate.

Determinations of the water and fat content were made upon groups of beetles as shown in Fig. 4. From an examination of these it is evident that during active feeding, the average water and fat content was 76.4 and 7.8 per cent. respectively. During *prehibernation*, the average water and fat content was 59 and

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[2] The term *prehibernation* is used to designate the quiescent state that occurs when feeding ceases and before the beetles burrow in the ground.

20 per cent. respectively. When the beetles burrowed in the ground the water content averaged 56 per cent. and the fat content 29 per cent. The results of these experiments indicate quite clearly that a loss of 20 per cent. in the water content and a gain of slightly over 20 per cent. in the fat content took place. Tower (24) obtained with this species a reduction of 27 per cent. in the water content. With hibernating grasshoppers Bodine (4) found a reduction of 13 per cent.

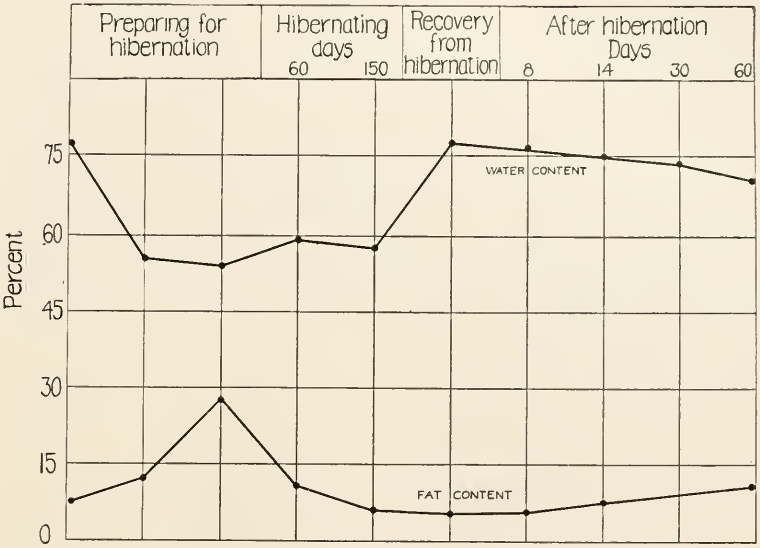

Fig. 4. Ordinates show the percentage of water and fat content of potato beetles, before, during and after hibernation. Note the reduction in the water content and increase in fat content as the beetles enter hibernation, and the extensive depletion in fat during the first few months as compared to the remaining period of hibernation.

The view commonly held is that a diminished water content makes protoplasm able to withstand extremes of temperatures. With potato beetles this does not appear to be valid, since invariably they must burrow in the ground to a considerable depth (10–18 inches) presumably to successfully escape extremes of temperatures. Experimentally the influence of greater variations in temperatures was determined by permitting only several inches of soil to intervene between the dormant beetles and the outside air. The results of such treatment upon hibernating beetles during the winter, produced a mortality of 100 per cent. In this species a reduction of the water content probably does not proceed to a sufficient degree to prevent injury by freezing temperatures, hence necessitating their further protection by burrowing in the ground.

During the progress of hibernation, continued investigations have shown that the water content remains practically constant, although with a general diminution of fat, a slight increase in the water content may follow. Extremely significant is the excessive depletion in fat that obviously takes place during the first two months (51 per cent.) as compared to the remaining five months (49 per cent.) of hibernation.

In this connection it is of interest to cite, for comparison, the results of other investigators concerning fat consumption during hibernation. Victoroff (26) with frogs found a consumption of 23 per cent. Investigators with mammals demonstrated the prevalence of a large fat content which gradually disappears during the period of torpidity. Thus Voit (27) found that in the marmot the adipose tissue was more than 30 per cent. of the weight of the body. Valentin (25) found the adipose tissue in the marmot contributed about twenty times as much food as the hibernating gland. He also observed that the depletion in fat which occurred during the first few months was greater than after five months hibernation. Similarly Carlier (8) found that almost one half of the fat disappeared in the hedgehog during the first few months of dormancy. It is of interest to discover this general conformity between a mammal and an insect in the utilization of nutrient material during the course of hibernation.

Hibernating Gland.

Many investigators mention a hibernating gland which is said to be commonly found in mammals (hedgehog, marmot, bat, shrew, rat, mole, beaver, squirrel, weasel, martin, badger, rabbit, guinea pig, cat, dog and even in man). Rasmussen (18) who has reviewed the literature on this subject extensively, is of the opinion that it is distinct from adipose tissue. Sheldon (22) recently concluded that "the so-called hibernating gland is essentially a form of adipose tissue which retains its embryonic characteristics for a more or less indefinite length of time, . . .

it intergrades with ordinary adipose tissue and under favorable conditions is transformed into it."

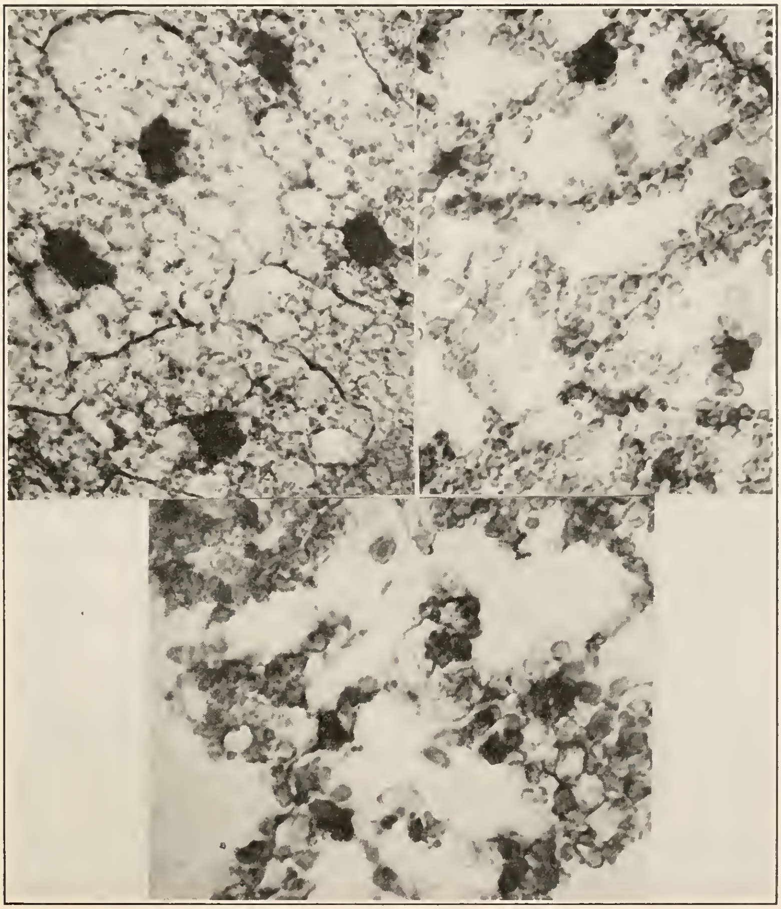

FIG. 5. Upper left. Section of the fat body or adipose tissue of potato beetle before hibernation. Note the numerous fat vacuoles partly masking the large nucleus located centrally within the cell, and the minute granules dispersed among the vacuoles. Flemming fixation, stained with hematoxylin. Photographed with achromat 2 mm., lens, 1.3 numerical aperture with 12 × compensating eye piece.

FIG. 6. Upper right. Section of the fat body after one month of hibernation, showing the decreased size of the fat vacuoles and nucleus, the large and prominent albuminoid granules grouped along the periphery of the cell exhibiting open spaces. Staining and enlargement as in Fig. 5.

FIG. 7. Section of the fat body after five months hibernation, showing the almost complete absence of fat vacuoles. The prominently large albuminoid granules are grouped in masses, the absence of cell outline of the fat body and the almost entire disintegration of the nucleus. Staining and enlargement as in Fig. 5.

Whether it is ordinary adipose tissue or a special gland that functions in the mammal as a storage for nutrient material, with the potato beetle, and perhaps with insects in general, it seems quite certain that adipose tissue serves that purpose. To determine some of the morphological changes that take place during hibernation sections were made of the adipose tissues of the potato beetle. During *prehibernation* (Fig. 5) the sections show it to be composed of regular cells, oval in general outline and filled with conspicuous fat vacuoles of more or less uniform shape and size which extensively overlap and partly mask the actual contour of the large nucleus. Barely distinguishable are diminutive albuminoid granules dispersed among the vacuoles. The sections of beetles in hibernation for one month (Fig. 6) are strikingly different in appearance from those of non-hibernating ones. In the former we find that the fat vacuoles have become much smaller and that the nucleus has undergone disintegration and diminution in size. The most striking objects are the albuminoid granules aggregated along the periphery of the cells of the fat body, or clustered around the nucleus. The sections of adipose tissues of animals in hibernation for the longest period (six months) show clearly the prominently large albuminoid granules clustered in groups, exhibiting many open spaces (Fig. 7). Indications of a dissolution of the cells of the fat bodies and a dispersal of the albuminoid granules are quite evident.

The author compared Figs. 5–7, with those published by Rasmussen and Sheldon of sections taken from the hibernating gland in mammals, and the similarity is indeed very striking.

The existence of albuminoid granules or uric acid concretions indicates an active metabolism in the fat body. According to Lang (15) the fat body of larvae of insects is rich in fat and poor in concretions of uric acid before metamorphosis, while in the adult the opposite is true. Fabre (11) thinks that the adipose tissue serves the purpose of a urinary organ, since urates are formed within the cells. Both Graber (13) and Landois (14) regard it as a single many lobed lung (owing to the many fine branched tracheal endings in the fat body). Similarly Roubaud's view of the necessity of a physiological purification during hibernation, based upon observing adipose tissue loaded with urate crystals before hibernation, must be regarded as signifying that it serves the purpose of an excretory organ.

There appears no valid reason for assigning to the fat body a urinary function. In the hibernating animal the metabolic activity of most cells is reduced to a minimum, and in the fat cells, judging from the respiratory quotient, it is most active. Hence the cells become charged with an accumulation of albuminoid granules and other products. The urates found in these cells are not an indication of their special urinary function, but of a more active metabolism at a period which serves the animal best. This may occur before or during metamorphosis, or throughout the progress of hibernation.

The Metabolism of Active, Starving and Hibernating Beetles.

A knowledge of the respiratory metabolism of active, starving and hibernating beetles seemed desirable for comparing the chemical changes involved. Of singular interest are the analogous rates of CO_2 output procured from beetles emerging from hibernation and pupation (Figs. 8–9), indicating, perhaps, a condition of physiological youngness or purification (Roubaud) as a result of hibernation. Bodine (4) found hibernating grasshoppers produced a higher rate of CO_2 output than growing animals kept at the same temperature, suggesting he states, "that animals remain young throughout the period of hibernation." The CO_2 output of potato beetles throughout the progress of hibernation invariably indicates a reduced metabolic activity, in some respects comparable with that of starving animals (Fig. 9). Indeed, there appears to be a parallelism in respiratory metabolism between starving and hibernating forms. In the former, however, the velocity of reaction of the life processes continues to function most actively and reserve substances are rapidly depleted, a condition eventually leading to the death of the animal. In the latter most of the life activities are considerably depressed and nutrient material is, therefore, used sparingly.

With older beetles, the metabolic activities are also extremely reduced, but not to the extent met with in hibernating or starving

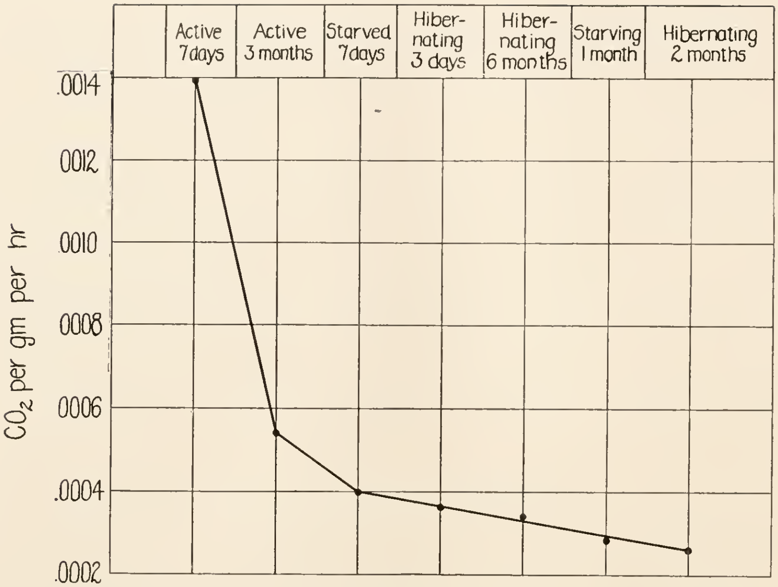

FIG. 8. Ordinates represent the CO_2 output per hour per gram body weight of organisms. Note the reduced CO_2 output of hibernating and starving as compared to active animals, indicating a parallelism in metabolic activity between starving and hibernating beetles.

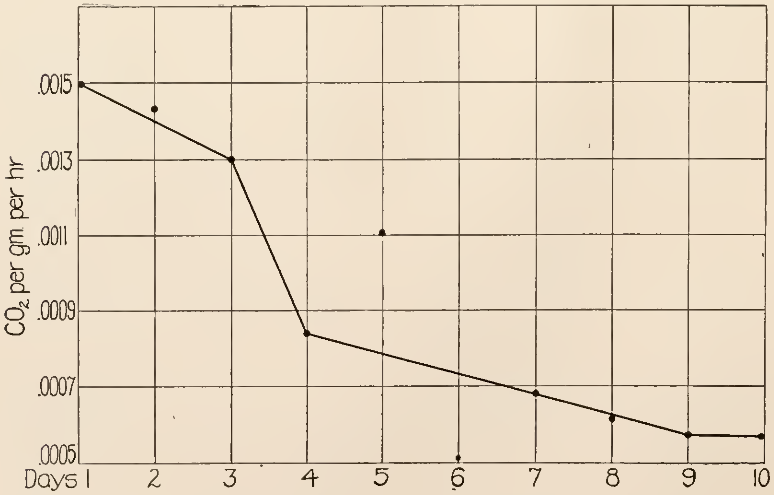

FIG. 9. Ordinates show the CO_2 output per hour per gram body weight of beetles during the progress of starvation until death. Abscissas represent days.

forms. The overfed and old state of the cell, as suggested by Baumberger (1) has perhaps reduced permeability to a wide degree. In old age or pathological conditions, according to Mathews (16), an accumulation of inactive ingredients or of acid in the cells, retards metabolic activity. In a measure, we are thus enabled to interpret the specific differences in metabolism that may take place under varying conditions in the organism. Especially noteworthy is the utilization of reserve nutrient material in the hibernating animal discussed further on.

Respiratory Quotient.

It is well known, experimentally, that when substances catabolized are chiefly fat, a reduced respiratory quotient results, whereas the catabolizing of carbohydrates and protein produces a higher quotient. According to the investigations of Dubois (10), Pembrey (17), Weinland and Riehl (29), Valentin (25) and others, reduced respiratory gas exchanges in the marmot occur during hibernation with strikingly low respiratory quotients (0.44–0.72). The views advanced to explain a quotient not consistent with the utilization of fat are many. Certain investigators (Dubois, Pembrey, Valentin) perceived that although mammals consumed no food during dormancy an increase in weight very often took place. It was, therefore, assumed that the oxygen consumed, greater than is necessary for fat oxidation, was in some way retained in the animal. Dubois also discusses building of acetone which he found accumulated in the blood-urine of hibernating mammals. It is known that of the normal fatty acids from butyric to decoic acid, only those with an even number of carbon atoms give rise to a marked increase in acetone formation. Dakin (9) and others have shown that acetone is derived from the decomposition of acetoacetic acid. This supports Knoop's theory of the B-oxidation in which two or some multiple of two carbon atoms are lost during oxidation, and offers a possible explanation that intermediate stages supervene throughout the process of the utilization of fat. Furthermore, Weinland and Riehl claim that during awakening from dormancy the marmot needs carbohydrates, and that the required carbohydrates come partly from substances produced in the body during hibernation. But it is not certain whether they are produced from fat, protein, or both. Voit (27) holds that fat may form sugar, which in turn can be stored up as glycogen.

The respiratory quotients obtained with the potato beetle demonstrate a wide degree of variation during different periods of hibernation. The respiratory quotient is lowest during the first few months of dormancy and increases progressively. A noteworthy increase in the quotients results when the animals awaken to activity (Fig. 10). This may agree with the theory brought forward by Weinland and Riehl, that carbohydrate is being oxidized for the purpose of awakening. On the other hand, during acute stages of starvation, potato beetles evince an exceedingly reduced respiratory quotient (0.54). Dubois has shown that a hibernating mammal loses as much in weight in 160 days as a starving animal in 12 days. Since it is recognized that reserve nutrient materials are rapidly depleted during starvation it is possible, perhaps, to correlate the diminished respiratory quotient noted during the early phases of hibernation

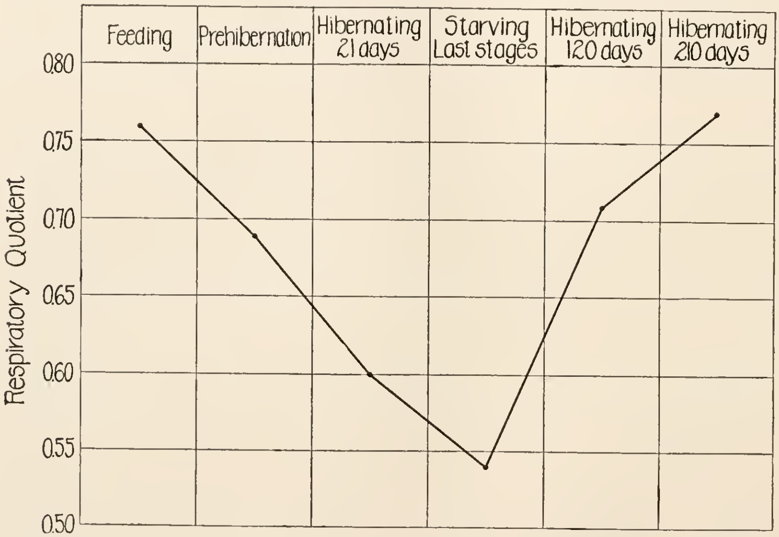

FIG. 10. Ordinates show the respiratory quotient based on respiratory metabolism (CO_2 output and oxygen intake) of active beetles and of those after varying periods of hibernation as compared to starving animals.

with the immense decrease in the fat content which likewise results at this period.

RECOVERY FROM HIBERNATION.

In the preparation for hibernation the potato beetles pass through a quiescent phase which has been designated as *pre-hibernation.* Likewise on emergence from dormancy there is a period of quiescence known as recovery. From results obtained on respiratory metabolism during recovery and before feeding, it is evident that the increase in CO_2 output is very gradual, lasting for six days (Fig. 11). The examples upon which the above figure is based, comprised a group of beetles in hibernation for six months. After varying periods of dormancy, if potato beetles are removed from the ground and placed with food, we find they require from six to thirty days for recovery before feeding. Those that have been in hibernation for over two months require 30 days, those for three months 12 days, and

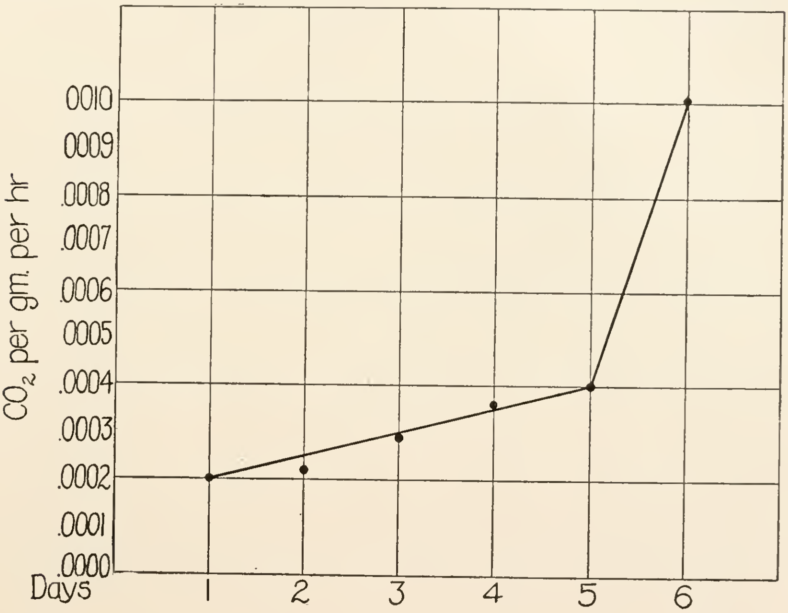

FIG. 11. Ordinates show the average CO_2 output per hour per gram body weight of beetles during recovery from hibernation. This figure is based on group of beetles hibernating for six months.

those five or more months 6 days. During the interval of restoration they are decidedly inactive, negatively heliotropic and do not feed.

Extensive experiments carried out during the winter with the view of hastening recovery proved fruitless. In every instance hibernating beetles when removed from the ground and placed with food persistently indicated tendencies to burrow themselves in again. In those cages not provided with soil the beetles remained inactive on the side or bottom of the cage without feeding.

Both Bodine (4) and Sanderson (20) found that the rate of recovery was quickest with animals brought in later in the hibernating period. Sanderson, however, concluded that subjecting hibernating forms to low temperature had the effect of producing a more complete rest than at a higher temperature. In view of additional experiments performed by the author a different interpretation may be attributed to the above results. For example, beetles reared in the greenhouse hibernated the latter part of March and were kept at a comparatively high temperature (65–95° F.) in the greenhouse. On July 11, they were found issuing from the ground in the cages, exhibiting normal activities such as feeding, mating, etc. In these experiments, therefore, cold was not essential for a complete rest. Furthermore, the depth at which potato beetles normally hibernate in the soil (10–18 inches) precludes the assumption of a specific influence of low temperature, or of the necessity of subjecting them to prolonged periods of cold. On the contrary, experimental evidence demonstrates a rhythmical period of rest irrespective of temperature.

Adjustment of the Water Content.

Of paramount importance to the animal during recovery and before feeding is possible, is an adjustment of its water content to normal. The need for actual contact of the animal with water at different intervals seems to be essential. Whether moisture may also be absorbed through the integument is not definitely known.

In the following experiments beetles recovering from hibernation were kept in cages with abundance of food under the same temperature conditions in the greenhouse. One lot frequently received water, the other lot was not watered. The addition of water (a drenching of the cage) had an activating effect, since the beetles eventually became active and fed normally on the plants. In those cages not receiving water the beetles were apparently unable to recover and finally perished. The lack of actual contact of the animal with water evidently hindered recovery.

Dissected hibernating beetles invariably revealed accumulated waste products in the rectum. In normal animals its expulsion is necessary before activity and feeding is possible. It seems reasonable that water actually is imbibed by the animal during recovery to aid in the elimination of inert substances from the digestive tract.

Catalase and Oxidase Activity.

It seemed desirable to determine if other factors concomitant with a lowered metabolic activity are involved in the hibernating animal. There are many facts in the literature tending to show that the power of decomposing hydrogen peroxide and the power to blue guaiacum by the aid of peroxide are the specific property of certain substances. Since the recognition of catalase as a specific enzyme, its occurrence and distribution in various animal and vegetable tissues has been investigated by a number of observers.

Certain investigators are of the opinion that the accumulation of hydrogen peroxide would undoubtedly prove harmful to the organism, and that the function of the catalase is to destroy the hydrogen peroxide as fast as it is formed. Others hold that the function of catalase may be to prevent the excessive oxidation of organic substances in the living cell. Schoenbein (21), however, has shown that substances which can bring about the decomposition of hydrogen peroxide catalytically, can also greatly increase its oxidizing power, and in proportion as a substance is able to decompose the peroxide so also it can accelerate oxidation. The power to decompose hydrogen peroxide is held by Spitzer (23) to be a measure of the oxidizing power of various animal tissues. Dakin (9), however, is of the opinion that there is no trace of evidence to prove that catalase is directly concerned with oxidation, since catalase, he states, "only liberates inactive molecular oxygen when decomposing hydrogen peroxide." Burge (7) found a quantitative relation between the amount of catalase and oxidation; an increase of oxidation in young and a decrease in old animals. Similarly Bodine (5) with certain insects obtained a decreased catalase content with increasing age and with animals subjected to starvation.

The author made determinations of the activity and total content of catalase in hibernating, starving, young and old potato beetles, using for this purpose the same methods described by Burge. The results of these experiments are graphically represented in Figs. 12–13. With hibernating animals the total catalase content per gram body weight is lower than with either young or old beetles; starving animals indicate the lowest catalase content. In Fig. 13, the catalase activity per gram body weight per minute is recorded and strikingly indicates a seeming parallelism in catalase activity between hibernating and starving animals. Moreover, the extremely reduced catalase activity indicates a correlation with a diminished respiratory metabolism in hibernating and starving animals.

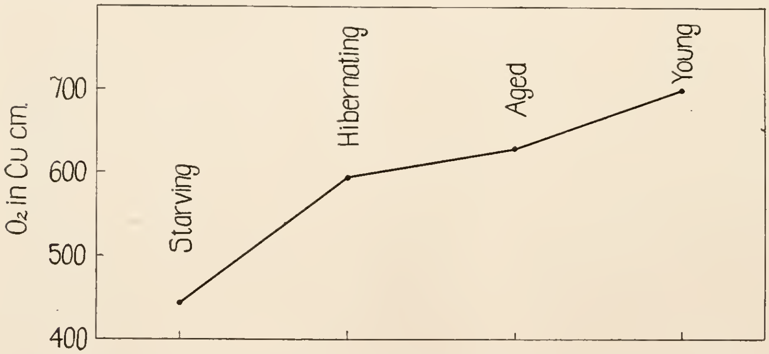

FIG. 12. The average total catalase content per gram body weight of different groups of beetles as measured by the oxygen evolved. Ordinates show the oxygen evolved in cubic centimeters.

Experiments to determine the oxidase activity were made by using guaiac, P-diamino benzene with peroxide upon tissues, organs and body fluids. Upon hibernating animals the reaction were negative since no typical characteristic blueing of guaiacum took place. Similar tests performed with active and starving beetles, gave striking reactions (blueing of guaiacum) with esophagus, stomach, tip of rectum, tissues, body fluids, testes and immature ova.

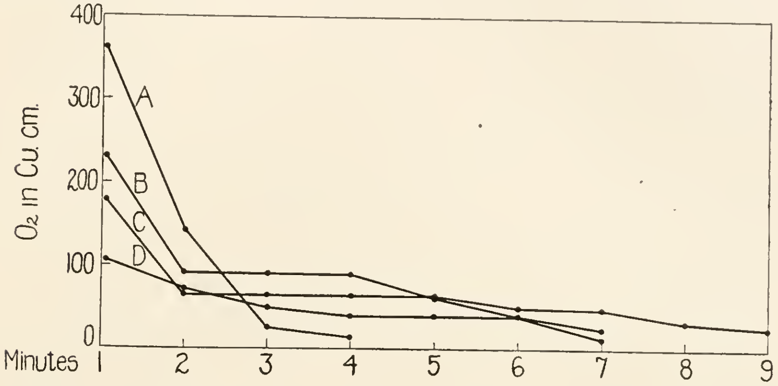

FIG. 13. The catalase activity per minute per gram body weight of organism. Ordinates show the oxygen evolved in cubic centimeters, abscissas as minutes. *A*, young beetles; *B*, aged beetles; *C*, hibernating beetles; *D*, starving beetles.

Although the catalases and oxidases may possess specific functions in the active animal, in the hibernating forms these enzymic activities obviously become greatly reduced or seemingly absent.

SUMMARY.

1. The results of the food experiments indicate that potato beetles of the first generation when fed on tubers and fruit of the potato, tomato, and eggplant require 13 days feeding to enter hibernation, while those fed on foliage of the same plants need 16 days. Beetles fed on potato foliage for six days followed by a non-solanaceous food required 23.6 days feeding before hibernation, and produced a mortality of 55 per cent. Those fed on pepper plants or on non-solanaceous foods do not hibernate, but die of starvation.

2. Respiratory metabolism determinations show that the oxygen consumption of beetles fed on tubers or fruit of solanaceous plants is less than of those fed on foliage. When fed on non-solanaceous foods, the reduced oxygen intake recorded was

comparable with the oxygen consumption of starving animals. A reduced CO_2 output occurs throughout the progress of hibernation.

3. Preparation for hibernation follows a period of extensive feeding and consists in an accumulation in the adipose tissues of 29 per cent. fat. During prehibernation a reduction of the water content of 20 per cent., the elimination of waste products from the digestive tract and a lowered metabolic activity of the animal take place.

4. The greatest depletion in fat occurs during the first few months of hibernation. In the metabolism of the fat body, the fat is replaced by albuminoid granules and other products. Sections of adipose tissue before and after hibernation are comparable to sections of the hibernating gland of mammals.

5. The respiratory quotient is lowest during the first few months of hibernation and highest at the termination of dormancy (0.60–0.76). In hibernating animals possessing insufficient nutriment and in starving animals, the respiratory quotient is exceedingly low (0.54). There appears to be a parallelism between a rapid depletion of reserve food and a low quotient.

6. Recovery depends upon the length of time potato beetles spend in hibernation. It is more rapid with those in hibernation for the longest time. Actual contact of the animal with water seems necessary to restore its water content before feeding is possible.

7. Catalase activity of hibernating beetles is greatly reduced and their total catalase content is lower than that for old or young active beetles. There appears to be a correlation between the reduced catalase activity and diminished respiratory metabolism in hibernating and starving animals.

Although active beetles give very striking oxidase reactions, no characteristic blueing of guaiacum was obtained with any organ, tissue, or body fluid of hibernating forms.

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BIOLOGICAL BULLETIN

ON THE FEEDING REACTIONS AND DIGESTION IN THE CORAL POLYP *ASTRANGIA DANÆ*, WITH NOTES ON ITS SYMBIOSIS WITH ZOÖXANTHELLÆ.

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In comparison with the extensive literature on the feeding reactions and the digestion in actinians, these phenomena in madreporarian polyps have been studied by a few authors only. Our knowledge of these phenomena is chiefly due to Carlgren (1905, *Caryophyllia*), Duerden (1906, *Fungia* and *Favia*), Carpenter (1910, *Isophyllia*), and Vaughan (1912, various West Indian reef-corals). In the following pages the results are given of an investigation on the feeding reactions and the digestion in the coral-polyp *Astrangia danæ* Ag.

The work on which this paper is based was made possible by a subvention of the Netherland-America Foundation, which enabled me to visit the United States for some months. It was carried on in the Marine Biological Laboratory at Woods Hole (Mass.) in August and September, 1924. I want to thank here the Director of this Laboratory, Dr. F. R. Lillie, for the opportunity I obtained to work some time at this institution.

The study of the digestion in *Astrangia* is meant as a base for the further study on the digestion in the polyps of reef-corals. As is well known, the polyps of reef-corals contain a large number of unicellular algæ, the zoöxanthellæ, which are located in the entoderm-cells. Although this association of coral-polyps with algæ is generally considered as a kind of symbiosis, there are very few statements on the advantages which both organisms derive from it. One of the ways to investigate this problem is the comparison of the physiology of a form with symbiotic algæ with that of a form without these organisms. Now *Astrangia danæ* generally is completely devoid of zoöxanthellæ, but during the time I worked in Woods Hole I obtained some colonies which were strongly infected with these algæ. The fact that my material consisted partly of polyps without zoöxanthellæ and partly of those infected with the symbiotic algæ enabled me to make a comparison of the feeding and digestion in these different kinds of polyps. As I disposed during the first half of my time in the Marine Biological Laboratory only of polyps without zoöxanthellæ, the larger part of my experiments were made with such uninfected polyps. Afterwards, when comparing the phenomena of digestion in polyps without zoöxanthellæ with those found in uninfected polyps, I could at least partly investigate the rôle of the zoöxanthellæ in the feeding of the polyps which are infected by these algæ.

I kept the polyps of *Astrangia* easily alive in glass-vessels with running sea-water, and at the end of a month they were in the same healthy state as at the beginning of the experiments. Some colonies even had enlarged in size by budding. The fact that *Astrangia* may be kept alive for some time in artificial surroundings was already recognized by Agassiz (1850), who kept his specimens alive by changing the sea-water twice a day. This author also gives a description of the structure of the polyps and states that they can be fed with fragments of molluscs. Besides this he gives some notes on the digestion in *Astrangia*. According to Agassiz the food at first remains for some time in the upper part of the gastric cavity before it descends to the lower part of this cavity where it is mixed with water and distributed over the internal organs of the polyp. As may be seen in the following pages these notes on the digestion are not completely in accordance with my observations.

The description of the general form of the polyps by Agassiz is very accurate. He states that there are three cycles of tentacles, those of the first cycle are of a larger size than those of the second, whilst the tentacles of the third cycle are somewhat smaller than the others. At each extremity of the laterally compressed mouth a tentacle of the first cycle is found. Agassiz discerned two varieties in the species: one with white polyps and another in which the polyps were pink or rosy.

An account of the chief peculiarities of *Astrangia danæ*, accompanied by figures, some of which represent the polyps in an expanded form, are found in the publications of Mrs. and Mr. Agassiz (1865) and of Dana (1890). Verrill and Smith (1874) also give a short description of the polyps of this species. They state that the animals are white and that the polyps in expansion rise high above the skeleton. These authors also note that the polyps will feed readily upon fragments of molluscs or crustaceans.

In the literature on the coral *Astrangia* I have not found any remark on its symbiosis with zoöxanthellæ. In the greater part of the colonies of this species found in the Woods Hole region all polyps are completely free of zoöxanthellæ. This was invariably the case in the specimens which I obtained from the piles of the wharf at Woods Hole, in those dredged in the harbour between Woods Hole and Nonamesset Island and in those dredged to the southeast of Nonamesset Island from about 8 fathoms. On the other hand the specimens dredged to the southwest of Falmouth (south of the oyster-pond) were always more or less infected with zoöxanthellæ. Some of the polyps of this locality had a dark brown color owing to the multitude of yellow algæ occurring in their tissues, others had various lighter shades of brown or were almost white, but a microscopical examination of a part of their tentacles or oral disk proved that they invariably contained zoöxanthellæ. It is a strange fact that this symbiosis of the polyp with yellow cells in the Woods Hole region is restricted only to the colonies found in certain smaller localities.

The polyps which are not infected by zoöxanthellæ are quite colorless and these are therefore especially fit for the study of the feeding reactions as foreign bodies can be seen through the transparent tissues of the animals. Usually the skeleton has a greenish or light-red color, which is due to the occurrence of algæ living in the skeleton. Then at first sight the polyps sometimes seem to have a pink or greenish hue by the transparency of their tissues. Probably these red or green-colored algæ are only different stages in the development of one species as their microscopical structure is very much alike. As yet I have no positive evidence that they belong to one of the forms described by Duerden (1905). In some colonies a compact mass of algal matter is obtained after decalcifying the specimens, in other colonies only sparsely distributed threads are to be found. In an allied species, *Astrangia solitaria*, the skeleton is also penetrated by boring algæ (Duerden, 1902). Besides these boring forms there is another alga which lives in the tissue of the polyps. Mrs. Dr. A. Weber van Bosse, who kindly examined the preserved material of these algæ, found that it represents a new species of the genus *Streblonema*.[1]

For different reasons the polyps of *Astrangia* are·especially fit objects for the study of their feeding habits. During the daytime they are usually fairly well expanded.[2] After being disturbed the polyps contract within the skeleton, but usually they will expand again in a very short time after the disturbance. Even when they are transferred from one glass-vessel to another they soon return to their expanded state. One of the further advantages of *Astrangia* in my experiments was that the polyps will ingest almost every particle which is offered to them, as well food as non-nutrient objects.

All the polyps of freshly-collected colonies of *Astrangia danæ* do not react in quite the same way on a certain stimulus, probably on account of the disturbance caused when they are collected. When, however, the colonies have been a few days in the laboratory under constant external influences they constitute a fairly uniform material for feeding experiments. Especially more or less hungry polyps are very susceptible to a certain stimulus, as is the rule in anthozoans. On the contrary well-fed polyps react very slowly on the same stimulus. Also in this respect *Astrangia* agrees with other anthozoans (cf. Jennings, 1905).

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[1] I am indebted to Mrs. Dr. A. Weber van Bosse for the following diagnosis of this interesting form:

"*Streblonema Willyae* n. sp.

Frondibus microscopicis in telum *Astrangiae danae* penetrantibus, compositis e filamentis sterilibus, irregulariter alterne aut secundatis ramosis, 2–5 μ latis, aggregatis, fasciculos prope superficiem hospitis formantibus. Chromatophoris taeniatis aut disciformibus, parietem cellulae non totius tegentibus. Sporangiis ignotis. Gametangiis cylindricis aut fusiformibus, singulis aut ramosis in filamentis plerumque terminalibus, interdum lateralibus, longis 60–120 μ, latis 8–10 μ; loculis uni- et pluriseriatis. Pilis desunt.

This new species of *Streblonema* is so far interesting as it grows in a coral, not in the skeleton but in the soft tissue of the animal. It has this mode of living in an animal in common with the genus *Endodictyon*, but the apical and above all the branched gametangia seem to indicate that its proper place is in the genus *Streblonema*."

[2] It is a well-known fact that the greater part of the madreporarian coral polyps are nearly always found in a contracted state during the day-time. Besides *Astrangia* there are, however, exceptions on this rule, *e.g.*, the polyps of *Fungia*, *Goniopora* and *Euphyllia* from the East Indian coral reefs, which are during daytime in a fully expanded state.

The chief results of my experiments on *Astrangia* are published in a preliminary paper (Boschma, 1925), in which especially the rôle of the zoöxanthellæ in the feeding of the polyps is compared with that in other anthozoans.

Feeding Reactions.

The reactions of *Astrangia* to food and other substances are in many respects quite similar to those found in other representatives of the anthozoans. Before the results of my own investigations on *Astrangia* a short summary of the literature on this subject is given below.

Pollock (1883) was the first to describe that sea-anemones may perceive the presence of food-particles in their neighborhood, as this causes the opening of the mouth and movements of the tentacles of the animals. Loeb and Nagel some years afterwards worked out the reactions to food and other substances in actinians more in detail. According to Nagel (1892, 1894*a*, 1894*b*) the actinians have a sense of taste, which is located only in the tentacles, and the food is brought to the stomodæum by muscle-action. Nagel (1894*b*) already stated that hungry actinians also ingest morsels of paper besides food-particles, whereas well-fed ones will only take food. The results of Loeb (1891, 1895) differed in some respects from those of Nagel. The phenomenon which was called by Nagel the "sense of taste" is according to Loeb a reaction to chemical stimuli. Loeb showed that the tentacles are not the only organs in which the response to chemical stimuli is located, he stated that also ciliary actions plays a part in the feeding of actinians. Moreover by cutting an actinian into two halves Loeb proved that a part of such a polyp devoid of its tentacles even takes food more readily than a normal individual.

Parker (1896) confirmed Loeb's results that ciliary action is one of the chief factors in the feeding of actinians. Parker found that the cilia of the stomodæum generally beat outwards, but when food substances come into contact with these cilia they reverse their movement. In subsequent papers (Parker, 1905*a*, 1905*b*) the same author has worked out this phenomenon in further details. He found that certain chemical substances can induce the cilia to reverse. A number of statements on the part of the tentacles and of the ciliary action in different actinians during the feeding reactions is described by Carlgren (1905). This author found that in some species of actinians the ingestion of food takes place chiefly by ciliary action (*e.g.* in the Protantheæ); in other forms, however, the tentacles are the chief organs for the capture and transportation of the food to the mouth (*e.g. Tealia*).

The fact that not all the individuals of a certain species of actinian react in the same way on the same stimulus, was demonstrated by Jennings (1905). The state of metabolism of the actinian is one of the most important factors determining the reaction to substances which come into contact with the tentacles or the oral disk. Thus hungry polyps often will ingest inedible matter, while well-fed ones will refuse the same material. Piéron (1906) also states that different individuals of one species behave differently towards the same stimulus.

Different species of actinians also behave in quite a different way. Some species of actinians in confinement would ingest only a very limited variety of food, *e.g. Eloactis*, which refused everything but living *Balanoglossus* and *Hydroides* (Hargitt, 1907). On the other hand many other species ingest besides food also indigestible material. Instances of this are recorded by Torrey (1905) for *Sagartia*, Fleure and Walton (1907) for *Tealia*, Parker (1896) and Allabach (1905) for *Metridium*. In the case of *Metridium*, Parker (1905), however, has expressed the opinion that the foreign matter (filter paper) was touched by the hands of the experimenter and therefore acted as a chemical stimulus. All of the above-named forms, as also *Actinia* (cf. van der Ghinst, 1906) are able to discern food-particles from inedible matter. The latter is usually refused and food particles are ingested.

Summarizing the data available in the literature and those of his own investigations Parker (1917) states that the different factors which are combined to bring about the feeding reactions in actinians are the following: secretion of mucus, ciliary action, the action of the neuromuscular apparatus of the tentacles, of the œsophageal cilia, and of the transverse mesenteric muscles. Only the muscular reactions are under the control of the animal as a whole.

The feeding reactions of *Alcyonium* are described by Pratt (1906). In this form the tentacles are the chief organs for the capture of the food. The species exhibits a definite choice in selection of the food, as eggs of fish and of *Galathea* are not digested, whilst the polyps readily feed upon planktonic organisms and flesh of different fishes.

There are only a few papers dealing with the feeding reactions in madreporarian polyps. The first species, in which observations on the capture of the prey are recorded, is *Astrangia danæ.* Verrill and Smith (1874) mention that it catches its food with the tentacles, which afterwards transfer the food to the mouth. De Lacaze-Duthiers (1877) made some feeding experiments with *Caryophyllia Smithii* and *Balanophyllia regia.* In the former species the food, a piece of a living mollusk, placed on the oral disk, caused a depression of the disk in this place. By the action of the muscles of the oral disk it was now brought to the mouth. The tentacles remained quite inactive. In *Balanophyllia* on the contrary the tentacles pushed the food towards the mouth. After some time in both species the food was discharged through the mouth covered with mucus. These statements, however, can hardly represent the normal behavior of the polyps. Probably the animals were in an abnormal state, for De Lacaze-Duthiers had kept them during several years in captivity. Moreover these statements are not in accordance with those by Carlgren (1905) on *Caryophyllia.* According to Carlgren the tentacles of this species catch food-particles and deposit them on the central part of the oral disk. By ciliary movement they now are transported over the oral disk to the stomodæum and swallowed.

The polyps of *Siderastrea radians* seize the food, according to Duerden (1904), with the tentacles. According to the same author (Duerden, 1905) mucus plays an important part in the feeding reactions of *Fungia* and *Favia.* On the surface of the polyps a mucous layer is secreted. Small organisms which come into contact with the oral surface of the polyps are imbedded in the mucus, which is afterwards ingested.

Carpenter (1910) described the feeding reactions of *Isophyllia.* The tentacles of this species catch small planktonic organisms. When a certain amount of food is taken by the tentacles the sphincter of the edge-zone of the oral disk contracts and the oral disk then forms a kind of roof over the mouth and the surrounding parts. In the superficial chamber formed in this way the digestion of the food takes place.

Vaughan (1912, 1919) studied the reactions to food in *Mœandra areolata* and many other West Indian reef-corals. The food is ingested through the combined action of ciliary movements, secretion of mucus, and the movements of the tentacles. In some species, *e.g.*, in *Orbicella cavernosa*, the mesenterial filaments are often protruded through the column wall and can catch food and even digest it whilst remaining outside of the gastric cavity.

With my feeding experiments on *Astrangia* I could confirm the statements of former authors that it will readily take food in captivity. When not overfed the polyps even will ingest almost everything which is offered to them. The food-objects which I used in my experiments were the following: crab meat (the muscles of the legs of the spider crab, *Libinia caniculata* Say.), fish meat, the soft parts of mussels, different species of worms (*e.g.*, *Naraganseta coralii* Leidy which lives burrowing in the skeleton of *Astrangia*, *Hydroides* and other polychæt worms), pycnogonids, small amphipods, larvæ of higher crustaceans, copepods, ctenophores, etc. Also juice of crab or mussel meat is readily ingested. Besides these food-particles indigestible objects are ingested by hungry polyps with the same avidity: coarse sand, powdered writing chalk soaked in sea-water, clumps of litmus, carmine and iron carbonate.

When crab meat or fish meat is mixed with some coloring matter (India ink, litmus, ammoniac carmine, neutral red) it is as readily taken as pure meat. This method enabled me to trace the way of the food in the internal organs after it had been ingested, the coloring matter was only used as an indicator.

These experiments with pieces of meat could not give results which represent the normal feeding reactions. In the gastric cavity of freshly collected specimens often remains of small organisms are found, especially appendages of small crustaceans.

To study the normal feeding reactions I therefore fed the polyps with planktonic organisms, chiefly copepods and larvæ of decapod crustaceans. It proved to be an advantage when the food was colored, then it could be seen through the transparent tissues when it was captured and swallowed. For this reason I used the method employed by Fischel (1908) for daphnids and copepods of fresh water. To the sea-water which contained the animals a few drops of a solution of neutral red were added by means of which the water obtained a light yellow color. The copepods and other organisms now absorbed the coloring matter and gradually acquired a bright red hue. Especially the different parts of the intestinal tract absorbed a great quantity of neutral red, but also immediately beneath the skeleton and in the appendages the coloring matter was stored. These colored copepods and larvæ of decapods are as readily taken by the polyps as colorless ones.

When some of these colored planktonic organisms are put into a small glass containing a colony of *Astrangia* with expanded polyps the capture of these animals may be easily observed. Every now and then one of the animals comes into contact with a tentacle of a polyp. Smaller copepods then as a rule are immediately captured, they seem to stick to the tentacle, undoubtedly by their being paralyzed by nematocysts. The tentacle then suddenly contracts more or less and bends over the oral disk in the direction of the mouth. The prey, however, is not brought directly to the mouth. The latter, with the central part of the oral disk, slowly increases in height as a conical protusion and this expanded part gradually moves in the direction of the prey. At last the tentacle with the copepod comes into contact with the mouth, the tentacle releases the prey and bends back to its original place. The captured copepod slowly slides down through the stomodæum into the gastric cavity, undoubtedly by ciliary action, and the mouth returns to the central part of the disk.

Larger copepods and larvæ of decapods, however, are not so easily captured. When they touch a tentacle they often swim away with a sudden jerk, the attack of the nematocysts seeming insufficient to paralyze them. Only when these animals bump heavily against a tentacle they are immediately caught. Their struggling movements cause a number of neighboring tentacles to move towards them, so that they finally become completely enveloped in a number of tentacles. Then they are slowly transferred to the mouth in the above described way, but they often remain struggling heavily, even when going down the stomodæum. Usually, however, the tentacles keep these larger organisms in the same position for some time, and only after they have ceased to struggle they are transferred to the mouth.

These experiments with copepods and other small crustaceans show that the tentacles instantly react when they are touched by a free swimming animal. The oral disk on the contrary is quite indifferent to the contact of these planktonic organisms. Sometimes a small floating copepod may be seen sinking down and falling on the oral disk of a coral polyp. It can remain there for some time without calling forth any reaction of the polyp. Afterwards it can swim away unharmed unless it happens to touch one of the tentacles, in which case it immediately brings forth the capturing reactions of the tentacle.

In the case of the feeding experiments with crab meat or other non-moving material of food the movements of the tentacles are very slow. In expanded polyps the small morsels of meat strongly adhere to the tentacles to which it is offered, and usually some neighboring tentacles also bend towards the meat, more or less enveloping it. The mouth with the top of the conically expanded central part of the oral disk moves slowly towards the prey and the tentacles push the meat downwards into the stomodæum. Some tentacles often protrude into the stomodæum, pushing against the food-material. The mouth then closes and the food slowly passes down into the gastric cavity.

Generally after the feeding the polyps partly expand by raising their column often considerably above the skeleton. The tentacles also remain in a more or less expanded condition. This expansion of the polyps generally takes place as well after the feeding of meat as after the feeding of free-swimming organisms. In this state the polyps are almost indifferent to mechanical stimuli. Whilst a hungry polyp quickly contracts when gently touched by a forceps the polyps which have just fed do not react on the same stimulus. A further peculiarity is that often some time after feeding air-bubbles are to be seen in the upper parts of the polyps, probably escaping from the prey which is being dissolved in the gastric cavity.

The reactions of hungry polyps of *Astrangia* to comparatively heavy objects, as diminutive pebbles and clumps of litmus, writing chalk or iron carbonate, are almost the same. These objects, when laid against a tentacle or on the oral disk, bring about the feeding reaction, consisting of the above described movements of the tentacles and the central part of the oral disk. The objects are almost invariably ingested and come closely in contact with the mesenterial filaments. The mouth closes after the ingestion and the polyp remains for some time, in my experiments varying between 20 and 95 minutes (usually lasting about 50 minutes), quite motionless. The first movement made after this time usually is the enlargement of the central part of the oral disk to a conical protusion. Then the mouth opens and the foreign object is seen moving slowly upwards in the gastric cavity. The underside of the object is in touch with the mesenterial filaments of the polyp, which seem to push against it. In the stomodæum in all probability the outward movement of the foreign object is caused by ciliary action, but often some mesenterial filaments are seen protruding in the stomadæum till the object has been removed from the inside of the polyp. In this way the non-nutrient paticles are soon out of the gastric cavity, chiefly by the action of the mesenterial filaments. The object falls from the mouth on the oral disk and slides down on one side. Here the tentacles bend downwards and then the object is completely removed from the polyp.

The foreign objects which have been in the gastric cavity for some time are covered with a thin mucous layer. Undoubtedly the mucus has here a protective function, to avoid that noxious particles come into contact with the tissues of the polyp. This function of the mucus is already described by Gee (1913) in *Cribrina*. In *Astrangia* especially on the clumps of chalk which are removed from the gastric cavity the mucus is clearly visible. Often other particles imbedded in this layer are removed with the non-nutrient object. In this way very small quantities of remains of food may be obtained from the gastric cavity without causing any injury to the polyp. I used this method to make out whether or not zoöxanthellæ were present in the gastric cavity of polyps the tissues of which contained these unicellular algæ.

Small pieces of filter paper rarely induce the polyps of *Astrangia* to feeding reactions. To avoid the absorption of organic substances (cf. Parker, 1905) the pieces of paper were not touched with the hands, but they were handled by clean instruments and put on the tentacles of a hungry polyp. For some time the small objects remain attached to the tentacles, to which they adhere more or less, but as a rule they soon fall down without causing the feeding reaction. In some cases, however, a few pieces of filter paper are ingested and afterwards, after about 50 minutes, removed from the gastric cavity in the above described manner. The difference in behavior of hungry polyps to comparatively heavy objects as small pebbles and pieces of filter paper in all probability is caused by the difference in weight. The heavy objects give a definite mechanical stimulus which immediately brings forth the feeding reaction, whilst the pieces of filter paper act as indifferent objects.

When carmine-powder soaked in seawater is distributed over expanded hungry polyps large quantities are ingested. There is no evidence of ciliary action on the oral disk, probably cilia are not to be found here. Soon after the carmine has dropped on the oral disk it is imbedded in mucus and large strains of this mixture of mucus and carmine can be seen gliding down the stomodæum through the inward beating of the cilia of the latter. Often a quantity of carmine is ingested by the cells of the mesenterial filaments, but it also occurs, especially when large quantities of carmine are present in the gastric cavity, that clumps of carmine mixed with mucus are removed from this cavity as non-nutrient particles.

The feeding reactions of *Astrangia* in general therefore consist chiefly of four actions of the polyps: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

DIGESTION.

In a great number of anthozoans, chiefly in actinians, the phenomena of digestion are at least partially investigated. The different authors who have worked on this subject do not agree in every detail, though the chief features of the digestion are fairly well known.

The principles of our knowledge of the phenomena of digestion in actinians and other cœlenterates are chiefly due to Metschnikoff and Krukenberg. Metschnikoff (1880, 1882) found that small food-particles are ingested by the entoderm cells of the mesenterial filaments in an amœboid way. They are imbedded in these cells and digested here (intracellular digestion). Claus (1881) maintained that the ingestion of foreign corpuscles in the mesenterial filaments of cœlenterates was already described by him before Metschnikoff made mention of this fact. But in Metschnikoff's papers the phenomenon of ingestion was described in quite a convincing form, whilst Claus' previous investigations (Claus, 1874) only contained some notes on the question.

Krukenberg (1880) proved that the mesenterial filaments of actinians are organs of digestion and maintained that digestion takes place solely against these organs. According to this author the mesenterial filaments have to come in contact with the food, then a secretion of digestive fluid takes place and the food-particles are more or less dissolved (extracellular digestion)[3]. Krukenberg (1881, 1882*a*, 1882–86) was not inclined to attribute such an important function to the intracellular digestion of small particles as described by Metschnikoff. He was convinced that the filaments secrete a digestive fluid, though he was not able to demonstrate this fluid in a free state in the gastric cavity. He even expressed the opinion that the enzymes of the captured animals could be used by the actinians for their digestion (Krukenberg 1882*b*, 1882*c*).

The investigations of Willem (1892, 1893) made it probably that besides intracellular digestion also extracellular digestion of the food takes place in actinians. The larger food-particles are more or less dissolved into smaller corpuscles by the secretion of a digestive fluid, and afterwards the small objects are ingested by the entoderm-cells as described by Metschnikoff. The ingestion of these small particles usually takes place in a zone of the mesenterial filaments in the neighborhood of the free edge, but after abundant feeding all the entoderm cells of the gastric cavity can ingest food, even those of the acontia. In the sea-anemone *Tealia* the soft parts of amphipods of the genus *Talitrus* are completely dissolved till only the bare skeleton is left (Willem, 1892). In siphonophores Willem (1894) found approximately the same phenomena: here also extracellular and intracellular digestion are present.

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[3] In the work of O. and R. Hertwig (1879/80) already the opinion is put forward that the mesenterial filaments secrete a digestive fluid. This was based only on the study of the histological structure of these organs. The definite proof of the digestive function of the mesenterial filaments is given by the physiological studies of Metschnikoff and Krukenberg.

According to Chapeaux (1893) in the gastric cavity of actinians a free digestive fluid can be demonstrated. The secretion of this fluid occurs when food comes into contact with the mesenterial filaments. When the food is ingested in the entoderm cells the reactions of the food-vacuoles is acid. In siphonophores Chapeaux found that 15–20 hours after ingestion of food colored with litmus the vacuoles still had a red color. Chapeaux concluded from this fact that the digestive enzymes of actinians and siphonophores acted in an acid medium. The same opinion also is upheld by Metschnikoff (1893), Mesnil (1909) and Roaf (1910). On the other hand Jordan (1907*a*) supposed that after the acid reaction in the vacuoles an alkaline one would follow; the final resorption of the food probably would take place in the latter stage. The enzymes of the anthozoans then would present more likeness to those of other groups of invertebrates, and act in about the same way as the trypsin of vertebrates (cf. also Jordan, 1907*b*).

A number of investigations on digestion in actinians have been made to show whether there is only intracellular digestion in these animals or if there also is secreted a free digestive fluid which dissolves the larger corpuscles into small particles which can be ingested by the entoderm cells. According to Mesnil (1901) no free enzyme is secreted in the gastric cavity: the digestion in actinians is exclusively intracellular. Jordan (1907*a*), however, showed that food which could not come into contact with the mesenterial filaments could be digested, and concluded that an enzyme causing extracellular digestion can be secreted by the mesenterial filaments. This opinion is also found in an article by Willem (1916), who maintains that extracellular digestion of the larger particles precedes the ingestion (phagocytosis) of the small particles, which are further dissolved by intracellular digestion.

The opinion of Biedermann (1911) on this question is in some respects an intermediate one between the two views dealt with above: he supposes that besides the intracellular digestion also the secretion of a digestive enzyme occurs. The reason why the enzyme is not found free in the gastric cavity (Mesnil, 1901) is according to Biedermann probably the following: only small quantities of digestive fluid are secreted by the mesenterial filaments, and only in immediate contact with the food.

Jordan (1913) upholds his original opinion, based on experiments with food packed in filter paper (Jordan, 1907*a*), which was digested in the gastric cavity of the polyps. This proves that the enzyme can easily penetrate through the whole of the food corpuscles and diffuse through every part of the gastric cavity where food particles are present. There is no special need for a contact of the food with the mesenterial filaments.

The principal organs of digestion in madreporarians are the mesenterial filaments, the same as in actinians. In *Cœnopsammia* (*Dendrophyllia*) Gardiner (1900) found a small crustacean in one of the polyps, supported by the mesenterial filaments, which indicates that it was being digested here. In the mesenterial filaments of *Flabellum* the same author (Gardiner, 1904*a*) found fat globules and diatoms and other algal matter in the cells of the portion next to the edge which is crowded with nematocysts. When remains of partially digested food were found in the gastric cavity of madreporian polyps, these were lying against the mesenterial filaments, as recorded by Carpenter (1910) for *Isophyllia* (*Mussa*) and by myself (1924) for *Favia*. Moreover strong evidence for the digestive function of the mesenterial filaments is given by Vaughan (1912), who states that these organs in *Orbicella* may protrude through the column wall and catch and digest the food outside the gastric cavity.

In other groups of Anthozoa there are also a number of statements on the digestive function of the mesenterial filaments. In *Pennatula* and *Virgularia* Marshall and Marshall (1882) found foreign bodies embedded in the cells of these organs. Wilson (1883) has observed the ingestion of food by the mesenterial filaments of *Leptogorgia*, and the same author found diatoms and other solid foreign corpuscles enclosed in the mesenterial filaments of alcyonid polyps (Wilson, 1884). The most elaborate researches on digestion in octactinians have been made on *Alcyonium*. According to Hickson (1901) the mesenterial filaments of this animal secrete a digestive fluid which dissolves the food. The latter is afterwards ingested by the entoderm of the gastric cavity. These conclusions were confirmed by Pratt (1906). After elaborate feeding experiments on *Alcyonium* and the study of the changes in histological structure before and after feeding in the polyps of this form Pratt came to the same conclusion: in alcyonid polyps extracellular as well as intracellular digestion occurs. Before feeding the gland cells in the stomodæum and in the mesenterial filaments are filled with a secretion, after feeding they are empty. Pratt concludes that this secretion is mixed with the food in the gastric cavity and causes the partial dissolution of the larger objects. Afterwards the small particles are ingested by the cells of the mesenterial filaments.

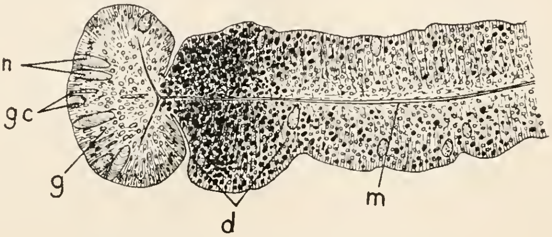
The opinion of Dantan (1921) that the digestion of antipatharian polyps is only extracellular is solely based on the histological study of the one polyp in which food-particles could be found. This observation does not give sufficient evidence for the opinion that the polyps of this group are in respect to their digestion quite different from all other anthozoans.

In the mesenterial filaments of zoanthids also foreign bodies are found embedded in the cells (McMurrich, 1889, 1899). The zoanthid polyps therefore in all probability digest their food in the same way as other anthozoans.

As stated above, a great number of investigators found that the mesenterial filaments are the chief organs of digestion in anthozoans. In many of these statements the accurate place of the ingestion is said to be the part immediately behind the marginal zone which contains a great number of gland cells and nematocysts. The mesenterial filament then consists of a marginal, glandular part and a part with digestive function next to the marginal part. Morphologically, however, the word "mesenterial filament" usually is employed in a more restricted sense, meaning only the marginal, glandular part (cf. Schneider, 1902). Also Duerden (1902), who states that the swollen part of the mesentery next to the marginal region is generally considered to be the principal seat of digestive function, denotes the marginal region only as the mesenterial filament. I use here the term in the same sense as other authors who studied digestion in anthozoans, and differently from the definitions of Schneider and Duerden. The glandular part of the mesenterial filament contains the bifurcation of the mesoglœa. It is sharply separated from the digestive part by a deep furrow; the digestive part is, as in other madreporarians (cf. Duerden, 1902), not sharply separated from the rest of the mesentery, usually its thickness is slightly more.

In my studies on the digestion of *Astrangia* I chiefly used pounded crab meat thoroughly mixed with some coloring matter which served as an indicator after the food had been swallowed. A number of small colonies served as material for each experiment. I noted the time of feeding and studied the changes in the internal organs at various intervals by cutting open a polyp and examining a mesenterial filament under the microscope.

In the case of crab meat colored with India ink the food is readily taken by the tentacles and transferred to the mouth. Then it slowly descends through the stomodæum into the gastric cavity. By the transparency of the tissues it remains visible in the lower part of this cavity, where it is in contact with the mesenterial filaments. The polyps gradually expand more or less and often rise considerably above the skeleton (to about 15 mm., the tentacles also may stretch to the same length). When, after a very short time, the food is ingested into the mesenterial filaments these organs are visible as dark stripes through the wall of the polyps. Already one hour after feeding the digestive part of the mesenterial filament is crowded with small black spots (the particles of India ink) which indicate that the food with the coloring matter has been ingested here. Sparsely distributed black particles are also often found in the adjoining portion of the mesentery. When food in great abundance has been offered to the polyps these parts ingest a great deal of it; when only moderate portions of food are given nearly all the food is ingested in the zone next to the marginal part. During a few days the distribution of the black particles in the mesenterial filaments does not alter perceptibly (Fig. 1); afterwards (about 5 days after feeding) the black particles gradually disappear from the digestive part of the mesenterial filaments. On the seventh day after the feeding usually the black particles have completely vanished from the digestive organs.



Explanation of the Text Figure.

Transverse section of the free extremity of a mesentery of *Astrangia danæ*, circa 2 days after the feeding of the polyp with a mixture of crab meat and India ink. Fixation 5 per cent. trichloracetic acid; stained with hematoxylin Delafield, *d*, digestive region of the mesenterial filament, crowded with black particles; *g*. glandular part of the mesenterial filament; *gc*, gland cells; *m*, mesoglœa; *n*, nematocysts. × 360.

Crab meat mixed with ammonia carmine gave approximately the same results: during a number of days after the feeding the digestive zone of the mesenterial filaments then contains a quantity of red vacuoles.

Another series of feeding experiments was made with crab meat mixed with litmus. This mixture keeps its blue color whilst remaining in the gastric cavity, but after the food has been ingested in the mesenterial filaments the latter acquire a red color. This change of color is clearly visible in the living animal through its transparent tissues. Microscopic examination of a mesenterial filament soon after feeding proves that in the digestive zone a large number of red vacuoles of different size are present, whilst particles of litmus lying against the mesenterial filaments but not yet ingested have retained their blue color. Consequently the digestive vacuoles possess an acid reaction whereas in the gastric cavity this reaction is neutral (or weakly alkaline).

During two days the digestive vacuoles keep approximately the same color. After about 48 hours a few vacuoles have acquired a bluish hue. Gradually the number of the blue vacuoles increases and after about three days the majority of the vacuoles in the digestive zone of the mesenterial filaments are blue. At last there are only a few red spots left, whilst the great number of blue vacuoles remain till about seven days after the feeding. During the greater part of the time the color is evenly distributed in the comparatively large vacuoles, but towards the end of the alkaline period (on the sixth and seventh day after the feeding) the coloring matter is concentrated to small particles, which gradually disappear from the mesenterial filaments.

In the digestion of *Astrangia* we can therefore distinguish two periods: an acid one, lasting for about two days, and an alkaline period during the rest of the time. In the later part of this alkaline period the excretion of the undigestible particles occurs. The changes in the reaction of the digestive vacuoles make it highly probable that the absorption of the food takes place in the alkaline period only. The acid period then is an antiseptic one (cf. Jordan, 1907*b*), in which noxious microörganisms are killed. The authors who stated that digestion of actinians takes place in an acid medium (cf. Chapeaux, Mesnil) did not extend their experiments for a sufficient time, otherwise they probably would have found that also in actinians the acid reaction is followed by an alkaline one. As compared with protozoans these two periods last a very long time. In *Paramecium* and *Colpidium* after feeding there is an acid period of 5½ to 70 minutes which is followed by an alkaline period of 1 to 30 minutes (Nirenstein, 1905).

One of the arguments for the presence of extracellular digestion in actinians results from the experiments of Willem (1892) referred to above. In *Astrangia* I have employed approximately the same method to demonstrate the evidence for extracellular digestion. I therefore studied the digestion of large copepods by the polyps. The copepods were vitally stained with neutral red after Fischel's method. At various intervals after the feeding I opened a polyp and noted the changes in the mesenterial filaments and in the copepods. The results were the following.

Half an hour after the feeding in the digestive zone of the mesenterial filaments a number of red vacuoles are visible. Besides these vacuoles which have a fuchsin like hue (acid reaction), the remainder of the mesenterial filament, including the marginal part, has absorbed some neutral red which is unchanged in color. The copepod still has a deep red hue.

Gradually now the intensity of the color of the mesenterial filaments increases whilst the color (together with the muscles and other soft parts) is extracted from the copepod. At first the parts of the copepod which are lying against the mesenterial filaments lose their color, the distal parts of the appendages usually keep the neutral red longer than the central part of the animal. After two hours the mesenterial filaments are strongly colored, whilst the copepod is almost completely devoid of its soft parts and only the bare skeleton is left.

When the polyps are cut open to study the changes in the food and in the digestive organs the parts of the skeleton of the copepod usually disjoint. To avoid this the experiment can be modified in some respects. When a polyp, including the skeleton, is cut longitudinally into two halves, the digestive function of the mesenterial filaments may be studied under the microscope. The skeleton prevents the tissues from contracting strongly after the dissection and the mesenterial filaments remain clearly visible.

A copepod vitally stained with neutral red placed on the mesenterial filaments soon becomes partly enveloped by a few coils of these organs and the struggling movements of the animal soon come to an end. After some time (a quarter of an hour to half an hour) these coils withdraw from the food and then the digestive zone of their surface which has been in contact with the copepod has assumed a red color, thereby forming a striking contrast with the remaining parts of the mesenterial filaments. These colored parts, then apparently saturated with food, slowly bend away from the copepod and other coils of the same or a neighboring mesenterial filament take their place. The copepod remains intact as far as the external form is concerned till almost all of the colored internal tissues have been absorbed by the mesenterial filaments. Afterwards usually the skeleton falls into pieces but not before almost the whole of the internal organs are dissolved. After about three hours the copepod is nearly completely devoid of its soft parts and the more or less disjointed skeletal elements are removed from between the mesenterial filaments.

This experiment, which can be easily followed under the microscope from the beginning till the end, gives a strong evidence for the view that besides intracellular digestion there is in *Astrangia* a secretion of an enzyme which dissolves the food in an extracellular way. Whilst the external surface of the copepod still is completely intact the greater part of the internal organs are already dissolved and ingested by the cells of the digestive zone of the mesenterial filaments. This disintegration of the soft parts of the copepod, as long as it does not yet fall into pieces, is only possible when a digestive fluid penetrates into it.

Probably in *Astrangia* a number of different digestive enzymes may be demonstrated as in the case of other cœlenterates (cf. Bodansky and Rose, 1922). The most effective of these enzymes is undoubtedly one which is comparable to the trypsin of other animals. This we may already expect in advance as the digestive vacuoles during the later period have an alkaline reaction. Moreover in all lower animals in which the proteolytic enzymes are studied they have a trypsin-like function (cf. Jordan, 1907*b*). I have made no elaborate experiments on the nature of the enzymes in *Astrangia*, but the few enzyme preparations tested showed that a trypsin-like enzyme is the chief factor for the disintegration of the food. Owing to the polyps being small the mesenterial filaments cannot easily be separated from the other parts of the polyps. The suspensions were made by pounding the tissues (chiefly consisting of the mesenterial filaments, extracted from a number of polyps) with sand to a mash. The latter was diluted with sea water and preserved with a few drops of chloroform. To equal parts of tissue suspension a piece of crab meat (previously boiled to destroy the blood enzymes of the crab it might contain) was added. Tests, containing crab meat in sea water with chloroform, were prepared in the same time. After four days the meat was partially dissolved. No positive results were obtained with the biuret reaction; the reaction with ninhydrin, however, gave a definite purplish blue color, proving that the liquid contained amino-acids or peptids (cf. Howell, 1922). In the tests the crab meat was practically unaltered, the liquid gave only negative results with both reagents.

THE FOOD OF ASTRANGIA AND THE SYMBIOTIC ALGÆ.

Among my material there were a great number of colonies the polyps of which possessed numerous zoöxanthellæ in their entoderm. The feeding of these polyps therefore can to some extent be compared with that of reef-corals, which, as a rule, also contain large quantities of these unicellular algæ. On the other hand the polyps of *Astrangia* with the symbiotic organisms can be directly compared with those of the same species which are completely free of zoöxanthellæ.

In the literature there are comperatively few notes on the food of Madreporaria. According to the statements of many authors (cf. Duerden, 1902; Gardiner, 1902–03; Pratt, 1906; Walther, 1919) only very rarely remnants of food are found in the gastric cavity of madreporarian and also of alcyonarian polyps. Now the greater part of the researches on which these statements are based were made after the study of preserved material, and as I have already pointed out before (Boschma, 1924) this is at least partially due to the contraction of the polyps in the fixing fluid. When coral-polyps strongly contract the food-remnants which are in their gastric cavity usually are discharged through the mouth.[4] In living coral-polyps from the reefs in the East Indian region I usually found remnants of food in the gastric cavity embedded in mucus.

To ascertain the natural food of *Astrangia* I now proceeded in the same way and studied the specimens in the living state. As soon as the colonies were dredged I put them in sea-water on board the ship and left them undisturbed for about a quarter of an hour. Gradually the polyps now expanded more or less. After a mechanical stimulus (a slight touch with a forceps) they suddenly contracted, and at the same time the mouth opened widely. Usually then at the bottom of the gastric cavity a slight

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[4] Some years ago Dantan (1921) also tried to explain this lack of food in the gastric cavity of anthozoans by the action of the preserving fluids which cause contraction and emptying of the gastric cavity.

amount of foreign material was to be seen, which could easily be extracted with a small forceps, without any damage to the polyps. The contents of the gastric cavity of 20 polyps consisted besides of undeterminable matter (detritus) of the following foreign organisms or parts of these: living diatoms (found in 12 polyps), diatom scales (found in 9 polyps), parts of higher algæ, usually in a partially decayed state (found in 4 polyps), foraminifera (found in 1 polyp), spicules of sponges (found in 5 polyps), parts of the stalks of hydroids (found in 2 polyps), a living nematode (found in 1 polyp), a dead larva of a polychæt worm (found in 1 polyp), parts of appendages or segments of the body of different smaller crustaceans (found in 10 polyps), shells of small bivalve mollusks (found in 3 polyps). Often also nematocysts or parts of these occur among the food-remnants and in the polyps which live in symbiosis with zoöxanthellæ invariably also these yellow algæ are to be found in the gastric cavity. Only very few polyps, when examined immediately after being collected, do not contain anything in their gastric cavity.

It is an interesting fact that in those polyps of *Astrangia* in which zoöxanthellæ occur in the entoderm cells, these algæ are always found in the remains of the food in the gastric cavity. These algæ are here in different stages of decomposition, owing to their being digested by the polyps. In this respect the polyps of *Astrangia* possessing zoöxanthellæ agree closely with reef-corals, in which the symbiotic algæ are also found in a partially digested state in the mesenterial filaments.[5]

There are, in general, two opinions concerning the food of reef-corals. One of these opinions was first put forward in a number of publications by Gardiner (1899, 1902, 1902–03, 1904*b*). This author found that the zoöxanthellæ form a large proportion of the food of all reef-corals, and maintains that many species of these corals even feed entirely on their symbiotic algæ. In a later publication Gardiner (1912) states that zoöxanthellæ are largely eaten by the coral-polyps when they require food, and further mentions that it is supposed that they catch and digest the small organisms of the superficial water of the sea. Hickson (1906) admits that the zoöxanthellæ may constitute a part of the food of reef-corals, but thinks it improbable that there are coral-polyps that feed exclusively on their yellow cells. In a recent work of Hickson the opinion is upheld that probably "the holozoic method of nutrition of the coral is supplemented by the holophytic action of the chlorophyll-bearing zoöxanthellæ" (Hickson, 1924, p. 21).

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[5] After my studies in Woods Hole I made some observations on the feeding of a few reef corals in the Bermuda Biological Station for Research. The polyps of these corals (*Isophyllia* and *Siderastrea*) in the natural state invariably contain partially decayed zoöxanthellæ in the digestive zone of the mesenterial filaments.

The other opinion on the food of reef-corals is expressed by Vaughan (1912, 1919) after a great number of feeding experiments on West Indian reef-corals. His conclusion is that the food of reef-corals solely consists of animal matter. This view is also supported by Mayer (1918), who based his opinion on Vaughan's experiments and on the statement of Duerden (1904) that the polyps of *Siderastrea* are easily kept alive with meat of crabs and other animals.[6]

Vaughan's opinion was first criticized by Gravier (1913). According to Gravier it is improbable that coral-polyps live exclusively on animal matter, for their symbiotic algæ are undoubtedly also a factor in their nutrition as direct food or indirectly by the supply of carbohydrates. After the study of the contents of the cœlenteron of many coral-polyps I also (Boschma, 1924) came to a conclusion differing from Vaughan's. I found that the food-remnants in the gastric cavity of the polyps of reef-corals always contained zoöxanthellæ in various stages of disintegration, undoubtedly owing to their being digested by the polyps. Besides these algæ also animal matter was found in the remains of the food, rarely in the smaller polyps, more often in the larger polyps.

It is generally understood that the products of the photosynthesis of the zoöxanthellæ assist in the nutrition of the cœlenterates containing these algæ (Buchner 1921, 1924; Hickson, 1924). In many anthozoans which harbor large quantities of these unicellular algæ even a degeneration of the food-capturing and digestive portions of the polyps has taken place, as in *Sclerophytum gardineri* (Pratt, 1903, 1906) and in *Galaxea musicalis* (Matthai, 1914).

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[6] Besides the facts on the food of reef corals there are some statements on the food of madreporarian corals which do not contain zoöxanthellæ in their entoderm. Some notes on the food of deep-sea corals are found in the publications of Gravier (1920) and Boschma (1924). In the latter article also data on the food of species of *Dendrophyllia* from shallow water are given. Cf. also Gardiner's articles on *Cœnopsammia* and *Flabellum*, cited above.

The feeding of cœlenterates on their symbiotic algæ as a whole has been reported in a few cases only. In young medusæ of *Aurelia aurita* Friedemann (1902) has mentioned instances of this phenomenon. According to Pratt (1903) in the polyps of the alcyonarian coral *Sclerophytum* frequently zoöxanthellæ are observed in a partially digested condition in the mesenterial filaments (cf. also Pratt, 1906). Digestion of zoöxanthellæ in the polyps of reef corals is recorded by myself (1924). Probably the irregular green corpuscles which McMurrich (1889) found among the zoöxanthellæ in the digestive part of the mesenterial filaments of zoanthids were also such partially digested algæ. In *Velella* the zoöxanthellæ of the developing larvæ which migrate to deeper water probably serve as a source of food-substance only, as their photosynthetic action is impeded by the darkness (Woltereck, 1904).

According to Fulton (1921, 1922) the association of cœlenterates with their zoöxanthellæ probably is of a parasitic nature (the polyps being the parasites of the algæ), as during starvation sea-anemones feed upon the unicellular algæ rather than upon their photosynthetic products.

Besides their feeding on the zoöxanthellæ or on their products of assimilation the polyps also derive some profit from the algæ living in their tissues, as they are a source of oxygen for the polyps. The investigations of Brandt (1883), Trendelenburg (1908) and Pütter (1911) prove that actinians with zoöxanthellæ may derive a large part of their oxygen from these algæ, and that actinians with zoöxanthellæ can better resist unfavorable circumstances than those which do not harbor unicellular algæ. Probably the algæ also use nitrogenous waste products of the polyps, which may be one of the profits the zoöxanthellæ derive from the association with the cœlenterates.

In the case of *Astrangia* zoöxanthellæ are always found in the digestive region of the mesenterial filaments of the polyps which are infected with these algæ. In contradistinction to the algæ in the entoderm of the oral disk and the tentacles those in the digestive zone have lost their yellow color to some extent. White spots then appear in their interior, whilst the contents are contracted and often have assumed a brownish hue. All different stages of the decomposition of these algæ are found in the digestive region, indicating the probability that they are being digested here. In the polyps of *Astrangia* which are not infected with zoöxanthellæ these algæ never occur in the digestive region of the mesenterial filaments. Consequently the zoöxanthellæ do not form a necessary part in the feeding of the polyps. A priori it is highly probable that they are digested on account of the lack of other food. We may therefore expect that, when food of other origin in great abundance is given to the polyps, the cells of the digestive zone of the mesenterial filaments will ingest no more zoöxanthellæ.

In fact these changes of the contents of the digestive region are easily accomplished. For my experiments I used dark brown polyps, the digestive organs of which before the artificial feeding contained a large quantity of partially decayed zoöxanthellæ. These polyps now were repeatedly fed with crab meat and already after three days the digestive region of the mesenterial filaments was almost completely devoid of zoöxanthellæ. Still a few yellow-brown corpuscles were recognizable as strongly decayed algæ, but it was evident that after the feeding of the polyps with crab meat no more algæ were ingested. The zoöxanthellæ which were already present in the digestive organs were completely digested. In this way the polyps can be compelled to change the nature of their food. It is obvious from these facts that the zoöxanthellæ are ingested by comparatively hungry polyps only. In the natural state the amount of food available for the polyps seems to be rather scanty and therefore they usually derive a part of their food from the digested unicellular algæ.

Van Trigt (1919) has described a similar phenomenon in fresh-water sponges (*Spongilla*), which contain zoöchlorellæ. These unicellular algæ, which are continually imported from the surrounding water, constitute a very important source of food for the sponges. When circumstances are favorable the algæ are killed and digested by the sponge only in part, the rest of the imported algæ then can live on, photosynthesize and multiply in the tissues.

On the other hand in less favorable circumstances the whole of the imported algæ together with those already present in the tissues of the sponges are digested.

Infection of Colorless Polyps with Zoöxanthellæ.

In my experiments I kept the colonies of white polyps separated from those with brown polyps (brown on account of their zoöxanthellæ). As the white polyps did not become infected during a month's time there is little evidence that infection occurs through free zoöxanthellæ in the sea water. In a number of polyps I produced an artificial infection with zoöxanthellæ by feeding them with portions of the soft parts of dark brown polyps. Especially those parts of the brown polyps which contained a multitude of these algæ (the tentacles and the oral disk) were used for this experiment. The tissues were torn to minute pieces and thoroughly mixed with pounded crab meat. It was necessary to add meat to the tissue preparations for otherwise the polyps refused to feed upon it.

Soon after feeding a number of zoöxanthellæ had detached from the crab meat and were floating free in the gastric cavity of the polyps and sometimes even the tentacles assumed a light brown color by the algæ which had penetrated into their cavity. A large part of the zoöxanthellæ from the feeding mixture were ingested together with the meat by the mesenterial filaments. After three days the digestive region of these organs was crowded with algæ. Some of these were already partially digested, for white spots had appeared in the originally evenly yellow colored interior.

Some of the cells which a very short time after the feeding are found in the cavity of the tentacles penetrate into the entoderm cells of these organs. Here they quickly multiply by division into two halves. The following numbers clearly illustrate the quick increase in number of the zoöxanthellæ in the tentacles of recently infected polyps. In one polyp on the day after the infection a cut-off top of a tentacle contained 6 symbiotic algæ, three days after the infection a top of a tentacle of the same length contained 23. For another polyp the number of zoöxanthellæ in the entoderm of different distal parts of tentacles of about equal size was: 1 day after the feeding 4 and 5; 2 days after the feeding 9, 16, 2, 21 and 12. In a third polyp the number of the algæ in tentacular extremities of approximately equal size was: 3 days after the feeding 30, 28, 22, 17 and 27; 9 days after the feeding 72 and 53. Consequently the zoöxanthellæ in the entoderm cells after the infection gradually had increased in number, though the change in color of the polyp could not yet be observed at first sight, for in moderately brown polyps extremities of tentacles of the same size as those cut-off in my experiments contain far more than 1,000 zoöxanthellæ.

After the feeding with the mixture of crab meat and zoöxanthellæ the infected polyps were fed several times with crab meat to keep them in a well-fed state. In hungry polyps the zoöxanthellæ perhaps might divide less rapidly.

I could not continue these experiments of infection for more than 9 days, but I am convinced that in this way a white polyp may be changed into a brown one crowded with zoöxanthellæ in the entoderm cells. Probably in nature the infection may take place (though rarely) in a similar way. When the polyps capture an animal which contains zoöxanthellæ some of these may find their way to the entoderm of the tentacles or the oral disk. After division these cells may infect the neighboring cells and at last the whole of the entoderm of the colony. This manner of infection in all probability only rarely occurs. As in other coral polyps with symbiotic algæ the greater part of the infected polyps of *Astrangia* undoubtedly have originated from planulæ which already obtained the symbiotic algæ before hatching. This view is supported by the fact that all the infected polyps of the Woods Hole region were found in a comparatively restricted locality.

Summary.

The feeding reactions of *Astrangia* in general consist of four actions: muscular action of the tentacles, muscular action of the central part of the oral disk, secretion of mucus by the oral disk, and ciliary action of the stomodæum.

The reaction of the food-vacuoles in the digestive region of the mesenterial filaments immediately after the feeding is acid; after about two days the reaction changes to alkaline. In this alkaline

period in all probability the digestion takes place. There is sufficient evidence that besides intracellular digestion a secretion of a digestive fluid (a trypsin-like enzyme) occurs.

In the polyps of *Astrangia* which contain zoöxanthellæ in their entoderm these unicellular algæ furnish a part of the normal food of the polyps: a quantity of these algæ are digested in the mesenterial filaments.

Polyps of *Astrangia* without zoöxanthellæ can be easily infected with these algæ by feeding them with crab meat mixed with parts of the tissues of strongly infected polyps.

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A STUDY OF OXYGEN METABOLISM IN *DROSOPHILA MELANOGASTER.*

M. R. CLARE.

INTRODUCTION.

Although *Drosophila melanogaster* is uniquely favorable as material for genetical studies, relatively few physiological investigations have been conducted upon this animal. Its small size has certainly been a deterrent to such studies; yet, with suitable apparatus, this feature is unimportant. To supplement the remarkably full record we possess for its genetical behavior physiological studies are especially desirable, and an attempt is made in this paper to show the practicability of investigations into the metabolism of this fly and also to illustrate the type of results which such study can be expected to yield. The investigation was undertaken primarily to determine to what extent degree of inbreeding may be reflected in metabolism.

Measurements were made of the oxygen consumption of *Drosophila* pupæ, and these proved admirably adapted to this purpose. At no time during pupal life except at its extreme termination are results complicated by muscular movements, hence standard metabolism alone is measured. Moreover, the fly is so amenable to conditions of laboratory culture that experimental pupæ are available at all times and can be grown under standard conditions.

The investigation was conducted at the University of Pennsylvania, for which privilege the writer desires to acknowledge his indebtedness to Doctor C. E. McClung. He is under special obligations to Doctor J. H. Bodine, who not only suggested the problem but was ever ready with helpful suggestions throughout the progress of the work. The stocks of experimental flies were kindly contributed by Doctors C. B. Bridges, H. J. Muller, L. E. Griffin, Chas. Zeleny, J. H. Bodine and R. L. King.

MATERIAL AND METHODS.

Eight stocks of "wild" *Drosophila melanogaster* were employed in the study. Three of these were caught shortly before the work was undertaken in localities removed from centers where flies are grown. These may be called the "non-inbreds." The remaining five stocks had been inbred for a number of years before being received and, as some of these are well known, the following table of sources may be of interest.

TABLE I.

SHOWING DERIVATION OF EXPERIMENTAL MATERIALS.

NON-INBREDS.

Designation.	Source.	Captured.
G.	Portland, Oregon	August, 1923
H.	Hellam, Penna.	July, 1923
B.	Rockaway, N. J.	July, 1923

INBREDS.

Designation.	Original Name.	Source.	Inbred Since
C–2.	"Ossining"	Columbia University	1921
C–1.	"Pt. Pleasant"	Columbia University	1921
F.	"Florida No. 5"	University of Texas	1918
I.	——	University of Illinois	1916
P.		University of Penna.	Many years

Owing to some preliminary difficulties, work was not begun until December, 1923, or until the non-inbred stocks had become inbred for several generations. Thereafter readings were continued, with interruptions, until December, 1924, when the experimental work was concluded. The chronological distribution of the work is without significance to our study and will not be entered into.

The pupæ of *Drosophila melanogaster* are so small that it is impracticable to make an extensive series of measurements of oxygen consumption on single pupæ, hence lots of 10 or fewer pupæ were used for each determination. Readings were taken over a period of 4 or 5 hours each day throughout the duration of pupal life. Data were collected for 160 lots of pupæ.

The stock flies were grown in mass culture in large quinine bottles and kept at room temperature. The experimental pupæ, however, were always the products of single matings. From time to time the flies in a culture bottle were removed and matings were made up from new flies as they appeared which were never more than 20 hours old. The pairs of flies were cultured in shell vials (about 9 cm. long by 2 cm. diameter) containing banana agar and were transferred each day to fresh vials so that the pupæ forming in a particular vial resulted from eggs deposited therein on a single and known day. A complete cultural record was kept which included figures for the sex ratios of the flies appearing in all of the vials in order that a check might be had on any conditions of metabolism attributable to sex peculiarities of the matings. It happened, however, that for all of the matings the distribution of the sexes remained normal. Some of the matings were cultured at variable room temperature, ranging from 21° C. to 25° C. about a mean of 23° C.; others were cultured in an incubator at a constant temperature of 25° C. It is necessary to stress this distinction, for upon it will be based a natural division of the data into two parts. Hereafter, the pupæ formed at room temperature will be referred to as of the "first experimental period," whereas those formed at 25° C. will be referred to as of the "second experimental period."

The banana-agar was prepared according to the usual method and while still liquid about 5 or 6 cc. of the material were introduced into each previously sterilized vial, which was provided with a cotton plug. Usually a sufficient number of vials was prepared at a time to supply requirements for two or three days and kept in a refrigerator while awaiting use. Before introducing a pair of flies into a fresh vial, a small amount of powdered Magic Yeast was dusted on the surface of the culture medium and on this was placed a disc of towel paper cut somewhat smaller than the bore of the vial. When a very limited amount of paper is placed in a vial, the larvæ developing therein pupate on the glass without "spinning" and therefore require a minimum of cleaning in preparation for use. They can then be removed quite readily from the glass without danger of injury with a small brush after a preliminary wetting with water.

Whenever possible, the first 10 pupæ appearing in a vial were used for a determination, but quite frequently only a smaller number could be secured. Each evening the vials for the several matings were examined and any pupa which had appeared unduly early was checked with a wax pencil in order that it might not be included among the experimental pupæ selected the following morning. Accordingly, the maximum age of pupæ on which determinations were made was 15 or 16 hours, a point which must be kept in mind.

In preparation for the first reading a lot of pupæ was first washed in water with a camels' hair brush, then treated with 80 per cent. alcohol for 2 or 3 minutes to destroy any adhering yeast cells, rinsed in water and dried on filter paper. After being weighed on a delicate balance they were placed in a cotton-lined basket and suspended in the oxygen-measuring apparatus. Between determinations each lot of pupæ was kept in an individual moist chamber with a piece of moist filter paper. Weighings as well as determinations were continued each day until development was so far advanced that there was danger of flies emerging. As a final step, record was made of the sexes of the flies which issued from each lot of pupæ.

Rates of oxygen consumption were measured with an improved form of the manometer of Krogh (1915), described by Bodine and Orr (1925). Six manometers were used, a single one only being used for a given lot of pupæ during the period of pupal life. During readings the manometers were placed in a water bath which was kept at a constant temperature. From day to day, however, the temperature of the bath varied in accord with the temperature of the room, but this fluctuation is not registered in the determinations as oxygen values are always reduced to 0° C. Calculation of rates has been made on the basis of oxygen consumption per minute of time and both per gram body weight and per single pupa.

The "Oxygen Curve."

The duration of pupal life is influenced largely by temperature. An index of this correspondence is afforded by figures for the number of days on which oxygen determinations were possible—even though such figures do not represent the actual duration of pupal life. During the first experimental period 4-day pupæ were predominant, 5-day pupæ occurring rarely and 3-day pupæ only to the extent of 7 per cent. At a temperature of 25° C., on the other hand, the percentage of 3-day pupæ was increased to 60 per cent., while 5-day pupæ did not occur.

During the course of pupal development certain readjustments are in progress which are reflected in rates of metabolism. In Fig. 1 the changes in metabolism are shown in the form of curves. Those for 4-day pupæ are derived from a single mating of the second experimental period while those for 5-day pupæ pertain to the first period. Each curve shows an initial fall after the first day of pupal life succeeded by an abrupt or gradual rise. The most instructive are the curves for 5-day pupæ in which the period of depression of metabolic rate is seen to continue from the second to the third day. The curves for 4-day pupæ are obviously abbreviations of these.

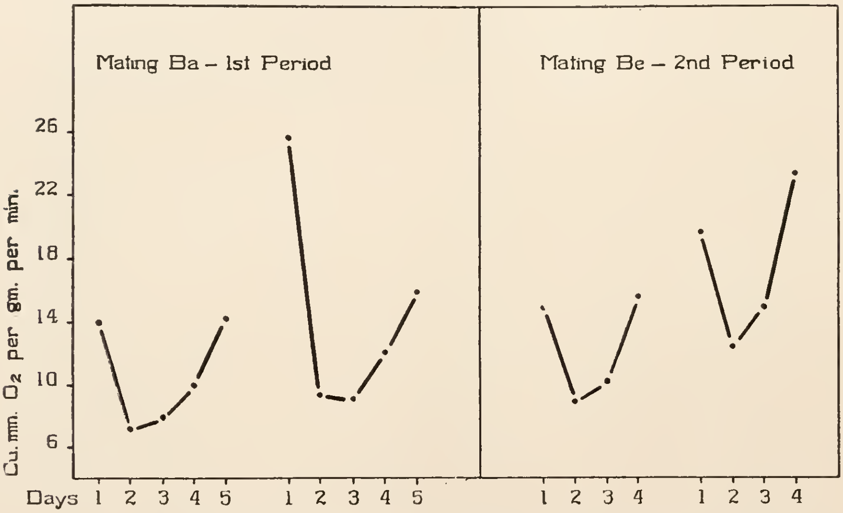

FIG. 1. Metabolism curves based on O_2 consumption per minute per gram of body weight. Ordinates = O_2 values in cubic millimeters; abscissæ = time in days.

This type of curve appears to be characteristic of pupæ in general, the modifications presented in the several species that have been investigated being due chiefly to the varying extensions of the period of depression. The significance of this type of pupal curve has been discussed at length by several workers among whom may be mentioned Tangl (1909, 1 and 2), Weinland (1906), and quite recently Fink (1925). The researches of Weismann, Perez, and others have demonstrated that early in pupal life the persisting larval tissues undergo a series of histolyses leading to their ultimate dissolution, and that the tissues of the imago are built up through the activity of certain groups of cells which survive histolysis and appear to be set aside for this specific purpose. In other words, two distinct processes are in progress during pupal life—a destructive and a constructive, the latter being inaugurated before the completion of the former. The authors cited identify the abrupt fall in metabolism early in pupal life with the histolytic process and the recovery after the period of depression with the formation and growth of imaginal tissues.

Variability in Rates of Metabolism.

When a series of curves for rates of oxygen consumption is examined, a feature which is most striking is the considerable variability in values exhibited. The majority of the values for any one day of pupal life fall within fairly narrow limits, but scattered among these are numerous others representing very high as well as rather low rates, distributed in a seemingly erratic manner. An early examination of the data proved that most of the very high rates belonged to lots of pupæ, one or more members of which not only failed to give issue to flies but failed to pass beyond the stage of development characteristic of second day pupæ. Instances of this sort suggest that probably intestinal microörganisms find their capacity for growth released by the death of the pupa or pupæ harboring them, and by their rapid multiplication elevate the rate of oxygen consumption to a high level. With one exception to be considered later, it was deemed necessary to completely eliminate from further consideration all data pertaining to lots of pupæ showing incomplete development, thereby reducing the total number of determinations by about one third. Several further eliminations are for lots of pupæ which were accidentally shaken from their supports within the manometers into the 2 per cent. NaOH solution over which they were suspended. Although washed as quickly as possible in a large volume of water and again set up in the manometer, a lot so treated almost invariably responded with an abnormal elevation of rate. Mention should also be made of a series of determinations which was set aside because of bacterial contamination of the cultures. This condition resulted in the production of a limited number of very small pupæ of exceptionally low weights. A single example from this series has been retained in Table 5 in order to show that, in spite of the very low pupal weights, the rate of metabolism is to all appearances normal. The rather drastic elimination which has been practiced has reduced to a considerable degree both the number of determinations and the variability in rates, but that the latter has been by no means removed is shown in Fig. 2.

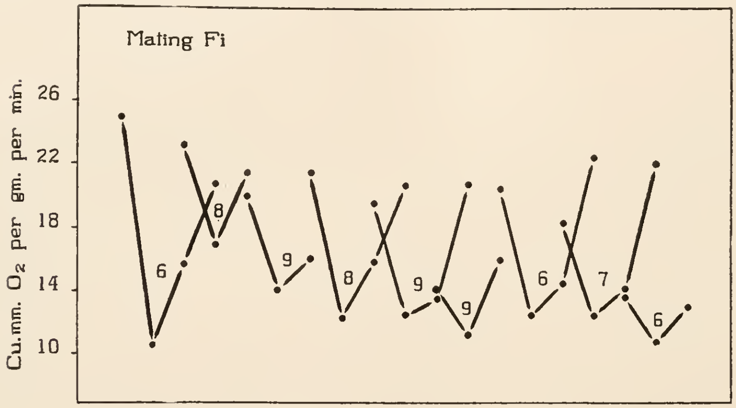

FIG. 2. Metabolism curves showing variability in rates within a single mating, cultured at 25° C. Numbers indicate particular manometers used in establishing rates. Ordinates = O_2 per gram per minute, in cu. mm.

There are several conceivable factors which might underlie this variability and they will be taken up in turn. It is obvious that if comparisons are to be made between the several stocks in respect to oxygen consumption this irregularity must be reduced to a minimum. Suspicion was at once cast on the calibration of the manometers used in making the determinations. That these are not the primary source of the variability will be evident from a further consideration of Fig. 2. The curves of this figure are for lots of pupæ all of which are offspring of a single pair of flies. Associated with each curve is a number which is that of the manometer used in determining the rates. For each of two manometers, numbers 6 and 9, three sets of determinations are represented which, it should be noted, exhibit marked variability in rates of metabolism. The manometers were very carefully calibrated several times and any possible errors from this source, although carefully looked for, have eluded detection.

Pupal Weights and Metabolism.

In studies on respiratory metabolism the practise has been to emphasize the relation between weights of experimental organisms and intake of oxygen or production of carbon dioxide, on the assumption that for comparable samples of living material within a species, the rates of metabolism remain fairly constant. Rubner, along with others, on the other hand, has instituted the procedure of basing metabolism on extent of body surface, arguing, in the words of Krogh (1916) that "the metabolism is simply a function of the conditions for loss of heat, while there is no such thing as a specific oxidative activity of the cell." Rubner later saw fit, however, to qualify this idea. These practises were derived from investigations on warm-blooded animals possessed of a heat-regulating mechanism, and their applicability to invertebrate animals is extremely uncertain. Unfortunately, the studies on metabolism in invertebrates have been so few in number and have afforded results of so conflicting a character that conclusions based on them do not seem to be justified. We feel obliged, therefore, to examine the data for *Drosophila* with some care and determine, if possible, the significance to be attached to weight; and attempt to decide whether or not rates of metabolism are subject to decided change. No data are available on which to base figures for pupal surface.

Pupal weights exhibit a wide range in value, the means for newly-formed or first-day pupæ in lots of 10 varying between 11 and 15 milligrams. At the extremes of the range lots of 10 pupæ may possess a weight as low as 9 mg. or as high as 17 mg. For the products of a single mating, likewise, the range of variability is very pronounced.

In addition to the rôles played by food and overcrowding as factors affecting pupal weight—and these can hardly be considered as applying in this work—temperature certainly is a determining agency. An examination of Table 2 shows that pupal weights of the second period are significantly lower than those for the first period. In the summer of 1923, during a

TABLE II.

PUPAL WEIGHTS AND OXYGEN PER GRAM FOR TOTAL STOCKS.

Figures for weights are based on lots of 10 pupæ, stated in milligrams; figures for oxygen consumption are rates per minute, stated in cubic millimeters.

	1st Day.	2d Day.	3d Day.	4th Day.
FIRST PERIOD—4-DAY PUPÆ.				
Weights	14.04 ± .20	12.57 ± .17	12.46 ± .17	12.36 ± .18
O_2 per Gram	15.89 ± .34	9.00 ± .15	10.38 ± .21	15.44 ± .42
SECOND PERIOD—4-DAY PUPÆ.				
Weights	13.30 ± .18	11.87 ± .15	11.78 ± .17	11.65 ± .18
O_2 per Gram	18.35 ± .44	11.00 ± .20	13.30 ± .35	21.15 ± .35
SECOND PERIOD—3-DAY PUPÆ.				
Weights	13.45 ± .18	12.25 ± .14	12.06 ± .14	
O_2 per Gram	17.94 ± .38	12.28 ± .28	16.71 ± .36	

period when the room temperature varied between 25° and 30° C., a collection of weights was made which for lots of 10 pupæ present mean values of 11.83 ± .152, 10.86 ± .169 and 11.00 ± .194 for the three days of pupal life. It is, therefore, evident that an inverse relation obtains between temperature and pupal weight. When the range of temperature is not very great,

TABLE III.

COEFFICIENTS OF VARIATION FOR PUPAL WEIGHTS AND OXYGEN PER GRAM.

The coefficients are based on the figures presented in Table II.

	1st Day.	2d Day.	3d Day.	4th Day.
FIRST PERIOD—4-DAY PUPÆ.				
Weights	11.2 ± 1.03	10.4 ± .97	10.4 ± .97	10.7 ± 1.02
O_2 per Gram	16.5 ± 1.51	13.0 ± 1.22	15.1 ± 1.41	20.3 ± 1.93
SECOND PERIOD—4-DAY PUPÆ.				
Weights	9.8 ± .97	9.1 ± .90	10.3 ± 1.02	10.2 ± 1.09
O_2 per Gram	17.3 ± 1.72	13.1 ± 1.30	18.5 ± 1.84	10.9 ± 1.16
SECOND PERIOD—3-DAY PUPÆ.				
Weights	11.3 ± .94	9.9 ± .83	9.4 ± .80	
O_2 per Gram	18.3 ± 1.52	19.2 ± 1.62	17.9 ± 1.53	

however, this correlation does not manifest itself when curves for temperature and pupal weights are compared. Moreover, a constant temperature serves merely to limit somewhat the range of fluctuation, instead of insuring stability, as can be seen from Table III. Temperature, then, operates only in a large way in its influence on weight of pupæ.

Corresponding to the sudden drop in metabolism shortly after the inauguration of pupal life, there occurs a marked drop in pupal weight. Sometimes this loss is relatively enormous, in other exceptional cases relatively insignificant. Unlike metabolism, however, there is no recovery in weight after the second day, the level for the third and fourth days remaining approximately identical with that for the second day. Sometimes for these latter days a slight increase is registered but as frequently a slight fall occurs.

The interesting sequence of changes during the pupal period in respect to pupal weights and metabolism is presented in Fig. 3. The data on which this figure is based represent a mating selected for illustration because a large number of determinations is available and the pupal weights show an unusual consistency of level. The figure is divided into parts corresponding to the several days of pupal life and a common scale is used which enables one to carry out comparisons between any two days.

The relationship between the curves for metabolism per pupa and per unit body weight affords an index of the ratio existing between weight of respiring tissue and that of inert materials associated with the respiring tissue. When the two curves follow a strictly parallel course this ratio must remain constant in the several lots of pupæ, provided complicating factors are not present. When for a particular determination the curves become approximated, the inference may be made that the relative amount of non-respiring or inert materials is reduced, whereas, when they become more widely separated, this condition may be attributed to a relative increase in the non-respiring materials present. It will be clear, therefore, that in general the ratio of respiring to non-respiring substances in the pupæ on which Fig. 3 is established remains a fixed quantity. Interesting exceptions, however, appear in the first and last determinations, numbers 8 and 37. In the latter the relation of the metabolism curves for the first day indicates that the excessive weight of the pupæ in question was due to the presence of a disproportionately great amount of non-respiring material, let us suggest water. As pupal development proceeded, this disproportion gradually became

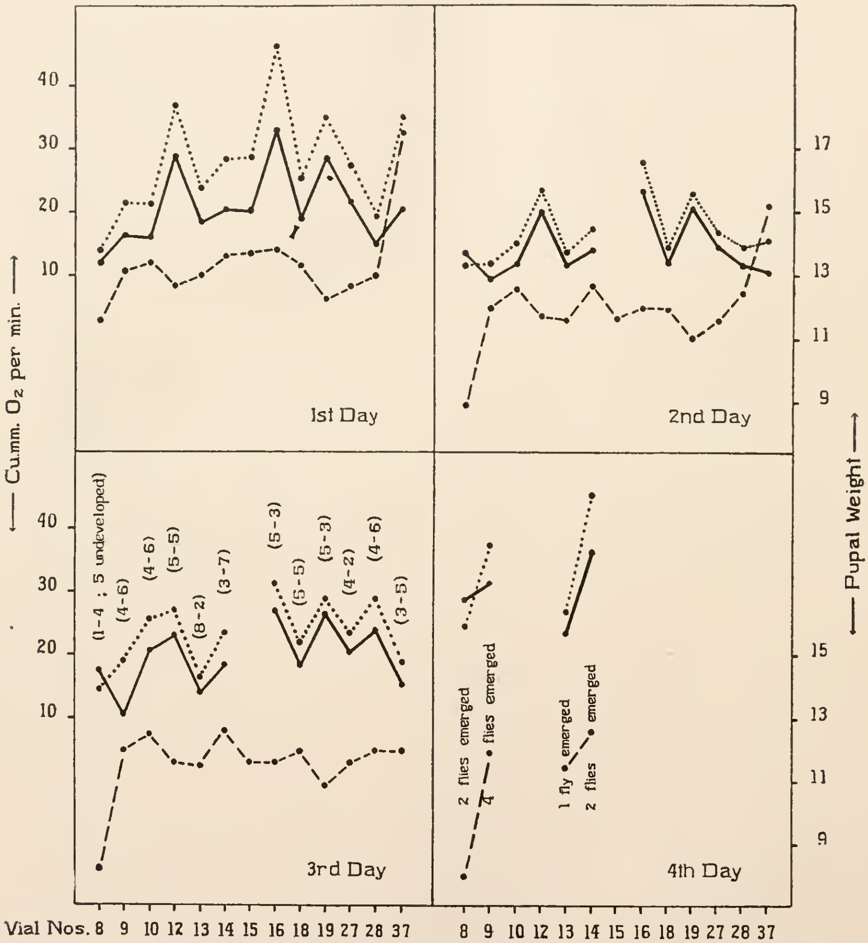

FIG. 3. Modifications in metabolic rates and pupal weights during pupal life for lots of pupæ derived from a single mating, C-2:4, at 25° C. Corresponding values for each day of pupal life are connected into curves. Upper curve = O_2 per pupa; middle curve = O_2 per gram and lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weights in milligrams per 10 pupæ. Abscissæ are numbers of the vials from which experimental pupæ were obtained. Sex-ratios in brackets (males—females).

reduced until, on the third day, the normal balance of respiring and non-respiring materials was restored. The first determination, number 8, on the other hand, presents a different story which is complicated by the fact that 5 of the 10 pupæ on which this determination is based failed to complete their development, the only case of the sort we shall consider. The approximation of the metabolism curves suggests that the pupæ contained a very small amount of non-respiring material relatively to the respiring tissues, yet this idea does not seem to harmonize with the remarkable fall in pupal weight occurring after the first day of pupal life. If, however, we postulate for this case, as was done earlier for cases of arrested development in general, a bacterial activity which would serve to elevate the metabolism per unit body weight relatively to the total metabolism, we develop an interpretation which affords an understanding not only of the peculiar relation shown by the two curves but also of the cause for the unusual decrease in pupal weight. Instances of this sort, representing a fluctuation in the relative amounts of respiring and non-respiring substances present in pupæ, are of frequent occurrence and are further illustrated in Fig. 5. The only general statement that seems permissible is that pupæ of very low weight usually show an elevation of the rate per unit weight relatively to the total metabolism but this condition is by no means invariably true. It appears, therefore, that mere weight is a rather unreliable index of the amount of respiring tissue.

It is manifest that for the first and second days of pupal life a rough proportionality certainly exists between pupal weights and metabolism, thus explaining in a way the trends of the oxygen curves. However, the peculiarities of the 12th, 16th and 19th determinations must receive an interpretation of their own. As the two metabolism curves remain closely parallel, the only conclusion that seems justifiable is that the rates for the determinations in question are of a discontinuous type; in other words, independent of pupal weight. It would appear, therefore, that strictly comparable samples of respiring tissue grown under, and subjected to, like environmental conditions can nevertheless exhibit marked diversity in rates of metabolism. It is exceedingly improbable that the independent rates of these determinations are due to a secondary source, bacterial for example, contributing in an additive manner to the respiring pupal tissues. If this were the case, we should expect the condition to be indicated by the metabolism curves.

As applied to *Drosophila*, the practice of bringing metabolism into relation with pupal weight is warranted only in a most general way. The correlation is most pronounced during the first day of pupal life when the coefficient of correlation for 4-day pupæ grown at 25° C. attains a value of 0.47 ± .071. On the second and subsequent days this value is considerably reduced. Too much importance should not be attached even to this correlation. The relationship often fails completely in individual cases, as is shown in Fig. 5. This fact, taken in connection with the rather frequent tendency toward the establishment of discontinuous rates, indicates that an understanding of metabolism in *Drosophila* is not to be sought on the basis of weight of respiring tissue but rather through the impress of factors regarding whose nature we are at present in ignorance.

A point of considerable interest brought out in Fig. 3 and in other figures for similarly prepared material is the constancy of the relation between the respiratory rates for the individual lots of pupæ up to the third day of pupal life. The striking similarity of the metabolism curves for the first and second days of pupal life indicates that the rates for this period are relatively stable and that the establishment of independent rates may occur either before or later but hardly during this period. On the third day, however, after the inauguration of growth and differentiation of imaginal tissues, a new order of rates is ushered in, which is that of adult life. As the metabolism curves for the third day in Fig. 3 show, these new rates considered in their entirety still preserve to a slight degree their kinship with those of early pupal life, but each lot of pupæ now develops along a new course. It appears that the rates of first-day pupæ in no way can serve to forecast those of the flies which will later emerge from them. We have already suggested that the period of institution of new adult rates is a somewhat critical stage in pupal development. The great majority of pupæ of arrested development, apart from those containing fully formed flies unable to escape from the pupal cases, represented the stage of development characteristic of 2-day-old pupæ. In other words, the histolytic process had been gone through but the reconstructive had failed of attainment.

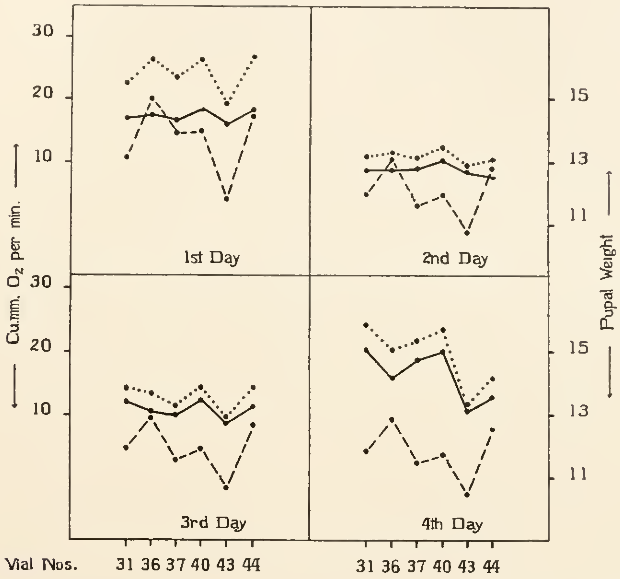

FIG. 4. Metabolic rates and pupal weights for pupæ of the first experimental period, products of single mating Hd. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates = O_2 values in cu. mm. at left, pupal weights in mg. per 10 pupæ at right. Abscissæ = numbers of the vials from which experimental pupæ were obtained.

Do flies about to emerge possess a higher standard metabolism than do larvæ? Our selected figure throws some light on this question. The elevated rates for the four-day pupæ in the figure are certainly in large part to be explained as due to muscular contractions incident to emergence of the flies, and accordingly these records do not indicate standard metabolism. When we recall that the figures for the first day of pupal existence are for pupæ which may be as old as 15 or 16 hours and in which histolysis and its accompanying depression in rate of metabolism has perhaps been in progress for an unknown length of time, it would appear from a comparison of the rates for the first and third days of pupal development that the standard metabolism of larvæ is actually somewhat greater than that of the flies although, relatively to body weight, the two stages may be more nearly comparable.

Before turning from the general subject we have been considering, a point of difference between the metabolism curves for the first and second experimental periods should be noted. For a cultural temperature of 25° C., we have seen that marked fluctuations occur in rates of oxygen consumption among lots of pupæ derived from a single mating, and corresponding in a very rough way with variations in pupal weight. In material representing the first experimental period at a lower temperature, the extent of fluctuations in metabolism is less pronounced until the last day when it becomes increased. Pupal weights of the first period are more irregular than for the second period and in consequence the parallelism between weight and metabolism tends to become lost. The relationship between pupal weight and metabolism for this period is represented in Fig. 4.

Influence of Sex on Metabolism.

It has been pointed out that for any one day of pupal life the metabolic rates for different samples of pupæ vary considerably and that weight is a very untrustworthy guide in arriving at an understanding of this irregularity. Is sex a contributing factor? Biological literature abounds in references to the physiological distinctness of the sexes and several important recent researches have depended for interpretation on the postulate that male animals possess a higher rate of metabolism than do female. So far as the writer knows, only one experimental attempt has been made to measure this supposed difference directly, namely, the investigation by Benedict and Emmes (1915). With human subjects these workers found a slight increase in metabolism in favor of males over the rates for females.

There are certain facts at hand to suggest a possible difference in metabolism between the sexes of *Drosophila*. At times, at least, there is a well developed tendency for female flies to emerge earlier than the males from the first-formed pupæ. The present data happen to be inconclusive on this point. The

TABLE IV.

SEX AND PUPAL WEIGHTS.

Pupal weights are stated in milligrams for lots of 10 pupæ.

First Day.

♂♂	Pupal Weights.										♀♀
	8	9	10	11	12	13	14	15	16	17	
1											9
2							×	14.30 ± 1.187			8
3							×	14.37 ± 0.780			7
4						×		13.35 ± 1.630			6
5						×		13.90 ± 1.337			5
6					×			12.87 ± 2.152			4
7					×			12.79 ± 1.778			3
											2
9											1

Second Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2						×		12.90 ± 1.300			8
3						×		13.00 ± 0.223			7
4					×			12.08 ± 1.245			6
5					×			12.38 ± 1.132			5
6				×				11.64 ± 1.679			4
7				×				11.58 ± 1.418			3
8											2
9											1

Third Day.

	8	9	10	11	12	13	14	15	16	17	
1											9
2											8
3					×			12.66 ± 0.145			7
4					×			12.00 ± 0.107			6
5					×			12.31 ± 0.175			5
6				×				11.54 ± 0.278			4
7				×				11.72 ± 0.213			3
8											2
9											1

records do, however, suggest a tendency for larvæ destined to become females to pupate earlier than those which will develop into males. Also, as is shown in Table IV., pupæ which are to become females incline toward heavier weight than those which become males.

The sex ratios for a typical series of determinations have been indicated in Fig. 5 as well as in Fig. 3. All of the data have been gone over most carefully with the unqualified result that sexual differences in metabolism are impossible to detect. If such exist they must be so small that they are obscured by irregularities induced by other agencies.

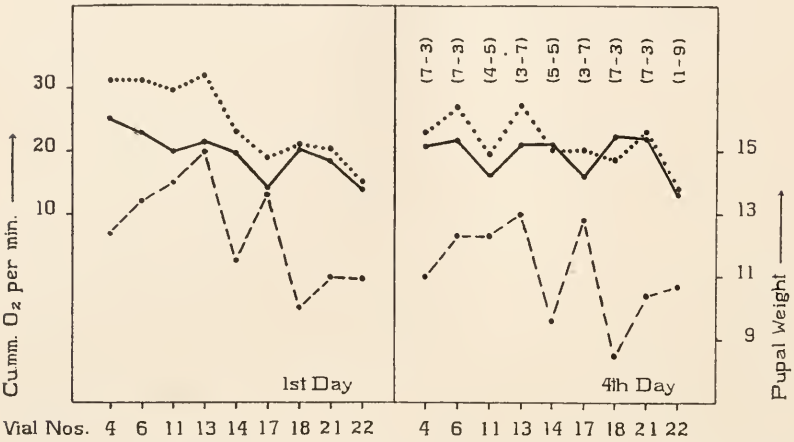

FIG. 5. Oxygen rates, pupal weights and sex-ratios for lots of pupæ of mating Fi, of the second experimental period. Curves for the second and third days of pupal life are omitted. Sex-ratios shown in brackets, the first figure for males, the second for females. Upper curve = O_2 per pupa; middle curve = O_2 per gram; lower curve = pupal weights. Ordinates at left are O_2 values in cu. mm., at right are pupal weight values in mg. per 10 pupæ. Abscissæ refer to the vials from which experimental pupæ were obtained.

FURTHER CONSIDERATIONS ON IRREGULARITY IN RESPIRATORY RATES.

The most significant result which this study has revealed is the peculiar irregularity in rates of metabolism exhibited especially by pupæ formed under conditions of constant temperature. It might be remarked that there is no ground for believing that the causes underlying this irregularity are genetic in character. On the contrary, the usual tendency of the rates for lots of pupæ derived on consecutive days from a common source to exhibit a graduated character either in the direction of elevation or of depression strongly suggests the effect of graduated environmental influences. We have already eliminated pupal weight and sex as important agencies responsible for irregularity in rates and we may now refer to several remaining possibilities.

It is believed that under the conditions of the experiment food conditions were maintained as nearly uniform as is possible. Moulds occasionally became established in the culture vials but the growth rarely if ever became noticeable until after the larvæ had pupated. It is altogether possible that organisms harmful to the larvæ and of sporadic occurrence in the tubes may have contributed in slight measure to the irregularity, but this is hardly a likelihood. We have referred to the bacterial contamination of one series of cultures, with the consequent reduction in weight of the pupæ. Curiously enough, this reduction in weight does not appear to have induced a parallel modification in rate of metabolism.

The pupæ of *Drosophila*, it would seem, are adapted to resist conditions of desiccation, at any rate for short periods. On several occasions lots of pupæ were inadvertently subjected overnight to the drying effect of the room air. This treatment produced no detectable effect on the following day either in pupal weight or in rate of oxygen consumption. This point is of some interest in connection with the results obtained by Caldwell (1925), who has found that the metabolism of some animals, as measured by carbon dioxide production, is modified as a result of desiccation.

A degree of irregularity in oxygen rates was certainly introduced with varying ages of the pupæ used. Larvæ developing from eggs deposited on a single day possess individual peculiarities, some pupating precociously, others delaying the act for a day or longer after their brothers and sisters have made the change. Accordingly, all of the pupæ thrown together for a determination are not of the same age. Among lots of experimental pupæ, however, the mean ages should be nearly identical as selection was performed at about the same hour each morning and the manometer readings were distributed over approximately identical time periods each day.

Mention might be made of an attempt to find an interpretation of the irregularity in rates through a study of varying intensities of such environmental factors as light, humidity, etc., data for which were secured from the local weather office. As might be expected, this attempt was not fruitful of results. Likewise, a careful examination of data on length of larval life, of pupal life, etc., proved barren of results, except that a suggestive but very rough correspondence between rates of oxygen consumption and productivity, as measured by the number of flies appearing in the vials, did come to light. A correspondence of this sort would carry the implication that the metabolic rates of the pupæ are due to an impress set by the metabolic condition of the female parent at or about the particular time the eggs are deposited, and that this impress continues without impairment over the period of larval life. The data at hand do little more than suggest this possibility.

Effects of Inbreeding.

The central question around which this study was planned was whether or not it is possible to find an index of inbreeding in rates of metabolism. If metabolism were subject to control as a result of genetic make-up, we should expect to find evidence of it reflected in our results. There is not only no indication that heredity plays any but the most general rôle in metabolism; but, on the contrary, we have seen some reason for believing that rates are at the command of graduated environmental influences. The irregularity in rates which we have stressed renders it impossible to make satisfactory comparisons between different matings within a common stock, and the propriety of lumping all of the matings of a stock under a mean is very questionable. However, this has been done in Table 5 in which are presented means for most of the stocks. The eliminations previously referred to made such serious inroads on the data for the majority of the matings that Table V. must be built up on an inadequate number of determinations. The figures for stock G of the first period are based on as few as 3 determinations; and in all other cases on 4 determinations except where a probable error is attached, this indicating that 5 or more determinations were available. Deficient as it is, this table illustrates the impossibility of separating inbred from non-inbred stocks on the basis of rates of metabolism.

Summary.

Several results emerge from this study which, it is believed, should assist in defining the problem and, at the same time,

Table V.

Pupal Weights and Oxygen per Gram (in Cu. Mm. per Minute) for Individual Stocks.

Non-inbred stocks are starred (*).

Stocks.	1st Day.		2d Day.		3d Day.		4th Day.	
	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.	Weight.	O_2 per G.
First Period—4-Day Pupæ.								
H*	14.25 ± .31	17.12 ± .42	12.62 ± .16	9.50 ± .18	12.62 ± .16	10.62 ± .29	12.62 ± .16	15.75 ± .73
G*	13.00	11.50	12.50	8.50	12.50	11.00	12.50	15.50
P	15.40 ± .15	15.20 ± .48	13.80 ± .12	8.60 ± .31	13.40 ± .24	10.20 ± .22	13.25	15.70
I	14.33	15.25	12.66	8.00	12.66	9.00	12.33	12.66
C–2	12.20 ± .35	16.40 ± .84	10.50	9.50	10.25	10.70	10.25	15.20
C–1	14.66	17.33	13.00	9.66	13.00	11.00	13.00	17.33
Second Period—4-Day Pupæ.								
H*[1]	11.71	17.42	10.71	9.71	10.40	11.75	10.66	17.00
G*	13.75	17.50	12.50	10.00	12.25	12.50	12.25	20.50
I	14.25	14.75	12.25	9.25	12.25	11.50	12.25	18.25
C–2	13.60 ± .15	19.60 ± .67	12.20 ± .12	12.00 ± .38	12.20 ± .12	14.00 ± .63	12.00	23.50
F	12.60 ± .33	19.30 ± .74	11.30 ± .28	11.60 ± .24	11.20 ± .34	14.60 ± .46	11.10 ± .31	22.10 ± .29

[1] The low weights of the pupæ of stock H of the second period were due to pollution of the cultures by bacteria.

should suggest types of investigation which are most likely to contribute to an understanding of metabolism in *Drosophila melanogaster*.

It has been shown that a close approach to a knowledge of metabolism in *Drosophila* pupæ cannot be made if sole dependence is placed on weight of respiring tissues as a guide. In a very rough way, pupal weights show a correspondence with the trends of metabolic rates, but, for particular matings, especially when the larvæ are maintained at lower temperatures, the correspondence is largely lost. It has also been shown that rates of oxygen consumption are very irregular and vary in a manner highly suggestive of the influence of environmental agencies. The not infrequently pronounced elevations above the general level of metabolism lend support to the conclusion that the metabolic rates for comparable samples of respiring tissue are not necessarily fixed within narrow limits. Finally, it has proved impossible to find a metabolic difference between the sexes or to detect any difference in metabolism between inbred and non-inbred stocks.

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THE ACTION OF ETHER ON PROTOPLASM.[1]

L. V. HEILBRUNN,

No problem in general physiology has been investigated more frequently than the problem of anesthesia. Many workers have attempted to discover the essential nature of the action of anesthetics on living substance. Not only have animals, tissues, and cells been studied, but some physiologists have even gone so far as to study the effect of anesthetics on lifeless materials which they believed similar to protoplasm.

Many theories have been proposed to explain anesthesia. In recent years the permeability theory has had a wide following. This theory claims that anesthetics either decrease the permeability of the cell, that is to say of the plasma membrane, or that they at any rate prevent an increased permeability. The actual evidence in support of the theory is somewhat scanty. What there is has been well summarized by Winterstein (1), Höber (2), and Lillie (3), all three of whom are strong advocates of the theory.

In only a few cases has it been shown that anesthetics lower cell permeability and most of these cases are doubtful, as Winterstein admits. In some instances observers have claimed that anesthetics produce an increase rather than a decrease in permeability (see Höber). Perhaps the work most often quoted in support of the doctrine that anesthetics lower permeability is the series of conductivity measurements of Osterhout (4) on plant cells. There is however a growing realization that a decrease in the electric conductivity measurements of a group of cells does not necessarily depend on a decrease in the permeability of the plasma membranes of these cells. Many factors play a part in influencing the conductivity measurement. When an electric current is sent through a mass of cells, certainly a large part of the current goes between and around the cells. Changes in the conductivity of the spaces between cells are therefore apt to be of greater moment than changes in the conductivity of the cells themselves. The factors involved include possible changes in the size and shape of the spaces between cells, as well as changes in their conductivity. One such factor is perhaps of especial importance for substances like the ordinary fat-solvent anesthetics which alter the viscosity of the medium in which they are dissolved. This is the effect of a change in the viscosity of a solution on its conductivity. Concerning this effect Walker (5) says: "The addition of a small quantity of a substance such as alcohol to water increases the viscosity of the water. Corresponding to this increase we find that the rate of diffusion is less when a substance is dissolved in water containing a little alcohol than the rate of diffusion when water alone is the solvent, no matter what the dissolved substance may be. Similarly the speed of ions in water containing alcohol is less than their speed in pure water."

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

Let us consider some of Osterhout's experiments more closely. He measured the conductivity of Laminaria in various solutions of anesthetics and compared it with the conductivity of the same material in sea-water. In one experiment he adds to 970 cc. sea-water 10 cc. of ether plus about 5 cc. of sea-water concentrated by evaporation until its conductivity was about double that of ordinary sea-water. In another experiment he adds to 970 cc. sea-water 30 cc. of absolute alcohol plus 15 cc. of concentrated sea-water (apparently made up as before), thus obtaining a 0.5 molecular solution of alcohol which he refers to as 0.05 molecular. This solution, according to Osterhout, has the same conductivity as sea-water. But this scarcely seems possible. Osterhout's solution has approximately the same concentration of salts as sea-water, but the viscosity of the solution is decidedly higher. Pissarjewsky and Karp (6) found that a 0.5 molecular concentration of alcohol lowers the conductivity of normal NaCl solution until it is about 8 per cent. below that of the conductivity of normal NaCl solution in pure water. On the other hand, the ether solution used by Osterhout would have, as he claims, approximately the same conductivity as sea-water, for Arrhenius (7) found that 1 per cent. ether lowers conductivity only about 2 per cent. for various types of electrolytes.

But there is also another important fact to be considered. Separating the cells of *Laminaria* is a network of cell walls. This is composed of cellulose and cellulose-like material which offers little resistance to dissolved substances and is obviously one of the main paths for an electric current. The adsorptive powers of cellulose are well known, although it apparently adsorbs electrolytes much more readily than non-polar compounds. In the finely divided condition in which the cellulose occurs in the cell walls it is not improbable that substances of low surface tension like ether, alcohol, and chloroform would be selectively adsorbed on it and more or less concentrated there. We should at least be led to expect this from Gibbs' adsorption equation. Moreover the surfaces of the cells would also tend to adsorb the anesthetic. If such adsorptive processes occur, then the conductivity of the cellulose framework as well as the conductivity of the cell surfaces would doubtless be decreased, and the decrease in conductivity would be greater than that which occurred in the mass of the solution. If this is true then it might in itself account for the variations in conductivity found by Osterhout.

It is interesting to note that the experiments of McClendon (8) on the diffusion of electrolytes from anesthetized pike eggs also fail to take cognizance of the direct effect of the anesthetic on the diffusion rate. It might be thought that the difference in diffusion rate would also explain McClendon's experiments. This is not the case. The presence of 2 per cent. alcohol would lower the diffusion rate less than 8 per cent. McClendon apparently found a lowering of as much as 50 per cent. in the total amount of chlorides diffused from the eggs. But his results are not as trustworthy as it might at first sight be thought. McClendon determined the diffused chlorides nephelometrically as silver chloride, precipitating them with silver nitrate. Now the precipitation of silver chloride varies under diverse conditions; there is a well known tendency for it to go into colloidal solution. The presence of alcohol either alone, or in conjunction with the albuminous substances also present, might act as a peptizing agent and hinder the precipitation of the silver chloride. Alcohol occasionally acts as a peptizing agent, and the precipitation of silver chloride is known to be hindered by at least one non-electrolyte (see Bancroft (9), pp. 167, 168). Richard and Wells (10) in their first description of the nephelometer stated that "care must be taken to have both standard solution and unknown solution subjected to precisely the same conditions, for varying conditions of precipitation may lead to differences in the appearance of the precipitate far greater than the possible optical error of the apparatus. Herein lies the chief caution to be noted in its use." Later this point was emphasized again by Richards (11), who points out that "if even moderately accurate analytical results are to be had with the nephelometer, the one essential point to be heeded is this: *the unknown solutions to be estimated must be treated in exactly the same way as the known standard solutions, which serve as the basis for comparison.*" This precaution was neglected by McClendon and his results are therefore unreliable.

McClendon's work and that of Osterhout constitute a large portion of the evidence presented by Winterstein and regarded by him as trustworthy evidence in favor of the permeability theory. It would seem therefore that the support of the theory does not always rest on very solid ground.

Most of the earlier work on permeability change during anesthesia concerned itself with the attempt to demonstrate a change in permeability toward dissolved substances. More recently both Winterstein (12) and Lillie (13) have independently shown a change in permeability to water. I have repeated both sets of experiments, although in the former case I performed only a few tests and my results can scarcely be considered as constituting either a proof or a disproof of Winterstein's views. Fig. 1 shows two examples of the entrance of water into normal and anesthetized muscle as shown by the increase in weight. The abscissas represent time, the ordinates the weight of the muscle. The muscle used was the gastrocnemius muscle of the frog, and a pair of muscles from a single animal was used in each experiment.

The curves show that the entrance of water is faster in the normal muscle than in the anesthetized muscle. Winterstein obtained a somewhat more striking difference; he used the sartorius muscle rather than the gastrocnemius.

As to the interpretation of the experiments, the difference in rate of osmotic flow may be due either to a change in the properties of the fluid or to a change in the plasma membranes. Bigelow (14) and Bartell (15) found that the speed of osmotic flow of water through various osmotic membranes followed the laws of Poseuille for flow through capillary tubes. It is obvious that in general any increase in the viscosity of a fluid would tend to slow its rate of osmotic flow. The magnitude of the effect might be sufficient to account for my results, although perhaps not for those of Winterstein. There are also other factors to be considered. The anesthetics may extract materials from the muscle which tend to exert osmotic pressure in the opposite direction.

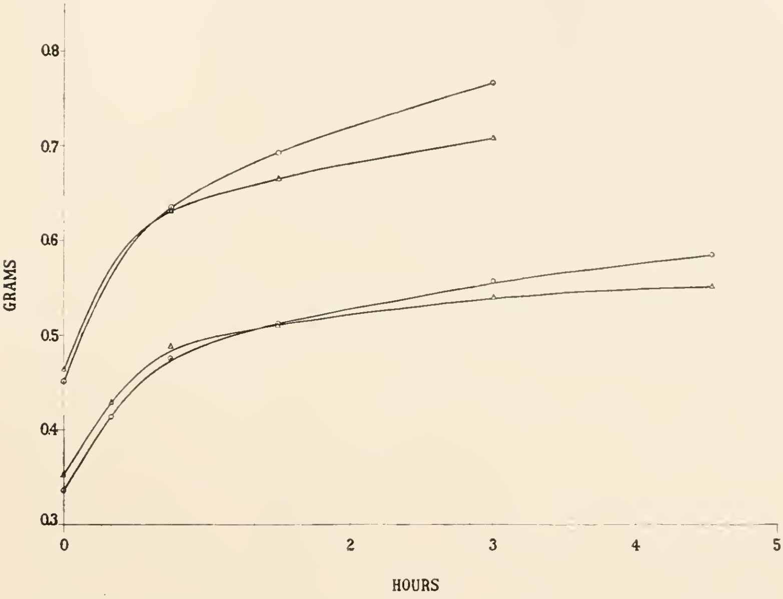

FIG. 1 shows the increase in weight of muscles in distilled water and in distilled water to which 2 per cent. by volume of ether has been added. In each pair of curves the small circles represent the increase in weight of a frog gastrocnemius muscle in distilled water, the small triangles represent the increase in weight of the corresponding muscle from the other leg immersed in 2 per cent. ether. The abscissæ indicate time in hours, the ordinates total weight of muscle.

I have thus far omitted reference to Winterstein's experiments on the osmotic flow through muscle membranes obtained from

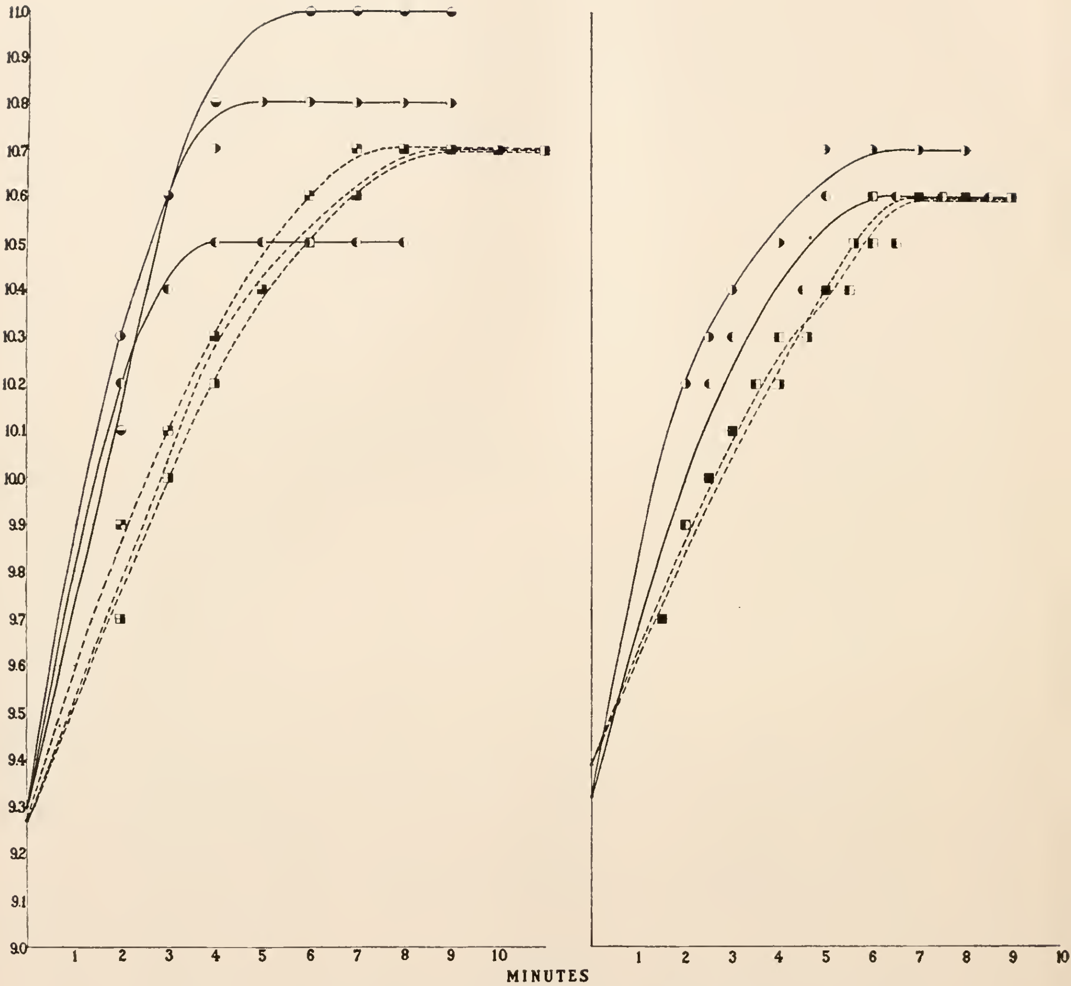

FIG. 2. In the above figures the curves represent increase of diameter of individual sea-urchin eggs in equal parts of sea-water and distilled water. (The original size is an average measurement.) The continuous lines represent etherized eggs, the broken lines control eggs, not exposed to ether. The concentration of ether used was 1 per cent. by volume, and the eggs were first treated with 1 per cent. ether in sea-water and were then transferred to 1 per cent. ether in equal parts of sea-water and distilled water. The ordinates represent the diameter of the eggs in arbitrary scale units, the abscissæ show the number of minutes elapsed after the entrance of the egg into the hypotonic solution. Similar results were also obtained with 2 per cent. ether solutions, but the measurements are harder to make as the eggs frequently rupture.

the body wall of female frogs. These experiments constitute the greater portion of Winterstein's evidence and the reader is referred to his paper for details. As I understand the experiments, Winterstein measured the osmotic flow into short glass cylinders sealed at *both* ends with muscle membranes. In such a system it would seem that the entrance of water would depend more on the distensibility of the muscle membranes than on their permeability. The high concentration of alcohol and other anesthetics used by Winterstein might tend to coagulate the membranes and make them less distensible. This would simulate a decreased permeability. However I have not worked with this sort of a set-up and I am not sure that I can judge it properly.

My results with sea-urchin eggs are perhaps more interesting. With this type of material Lillie found that the shrinkage of the cell in hypertonic solutions was delayed by the presence of ether in anesthetic concentration. This may very well be true, but it need not be due to a change in permeability to water. For it is easy to show that when eggs are placed in hypotonic solutions, they expand just as readily, or even more readily in the presence of ether than in its absence. This is illustrated in Fig. 2, which compares the expansion of etherized and unetherized eggs in hypotonic solutions. The shrinkage and expansion of a cell depends on many factors. Of these permeability is one, but not the only one. Other factors of importance are surface tension, the rigidity of the plasma membrane, and the colloidal condition of the interior. This last factor is of especial importance in Lillie's experiments. Lillie found that after fertilization the sea-urchin eggs showed a greater tendency to crenation and shrinkage when placed in hypertonic solutions, and that this increased tendency to crenation was prevented by anesthetics. As a matter of fact fertilized eggs become somewhat crenate even without being placed in hypertonic solutions (16). The crenation is concomitant with, and doubtless dependent on, the gelation of the protoplasm which follows fertilization. In view of the fact that anesthetics such as ether prevent this gelation (see below), it is easy to see that they would tend to hinder crenation.

Finally it should be pointed out that the experiments of Loeb (17) apparently indicate an *increase* in the permeability of *Fundulus* eggs to water in the presence of various anesthetics.

It is not my purpose to claim that in anesthesia there is no decrease in the permeability of the cell to salts or to water. Perhaps eventually this may be found to be a constant occurrence. But it seems at least premature to conclude a decreased permeability from the slight and uncertain evidence hitherto presented. Only by closing our eyes to the experiments that show the opposite of a decrease and only by neglecting the numerous sources of error which have scarcely been considered by previous experimenters, is it possible to consider the permeability evidence as convincing.

Even should we, some time in the future, find it true that anesthesia is always associated with decreased permeability of the plasma membrane of the cell, we would not be very much closer to an understanding of how and why the anesthetic stops cell activity. Permeability is a surface phenomenon, the activity of the cell goes on largely in the interior. Thus even if we accept the permeability doctrine, we must postulate a secondary hypothesis to explain how the permeability effect is transferred to the interior of the cell.

Many years before the permeability doctrine was thought of, various physiologists held the idea that anesthetics affected the colloidal condition of the protoplasm in one way or another. For a long time this idea remained a mere speculation, but in recent years it has been definitely shown that anesthetics do have a very real effect on the colloidal state, or at any rate upon the viscosity of the protoplasm. Both in plant and animal cells it has been shown that dilute solutions of ether cause a liquefaction of the protoplasm, and that more concentrated solutions cause a coagulation. There is a remarkable correspondence between widely different sorts of living substance. However in plants only those concentrations of ether which cause coagulation prevent the rotary movement of the protoplasm. This was therefore regarded by Heilbronn (18) as the anesthetic concentration. In animal cells on the other hand it was found that concentrations which caused liquefaction prevented cell-division and therefore acted as anesthetic (16). As a matter of fact there is no difficulty in reconciling the two sets of observations. For it seems certain that what is anesthetic for mitosis is not necessarily anesthetic for protoplasmic streaming. An increased fluidity of the protoplasm would doubtless increase the speed of streaming, and yet such a cell with rapidly flowing protoplasm would scarcely be able to undergo a mitotic division and would therefore be anesthetized so far as cell-division was concerned. This is essentially the view taken by Weber (19) who points out that there is full accord in the investigation of both plant and animal cells.

Doubtless protoplasmic activity involves frequent changes in colloidal condition. Presumably any agent which prevents such changes in colloidal condition without causing death may act as an anesthetic. If this view is correct then either an increased liquefaction or a coagulation of the protoplasm may result in anesthesia. This idea was presented a number of years ago, and it was pointed out that there may very well be two kinds of anesthesia (20).

In earlier work it was claimed that those solutions of ether which act as an anesthetic for the process of cell-division in the sea-urchin egg cause a liquefaction of the protoplasm (16, 20). More concentrated solutions were found to cause a coagulation which was irreversible. All these facts have recently been called into question by Chambers (21). He claims that anesthesia in the sea-urchin egg is accompanied by an *increase* rather than a decrease in viscosity and that this condition of increased viscosity is reversible. Chambers' opinion is based on observations of Brownian movement as well as on a study with the microdissection apparatus.

It is evident that the findings of Chambers are in direct opposition to the earlier work mentioned above. An attempt was therefore made to repeat the older observations with the centrifuge method to determine if perhaps an error may not have been involved. In this repetition an effort was made to obtain more nearly quantitative results.

Experiments were performed both with fertilized and unfertilized eggs. We will consider the unfertilized eggs first.

When (unfertilized) sea-urchin eggs are placed in 2½ per cent. or 3 per cent ether, there is, within a few minutes, a very pronounced decrease in viscosity as shown by tests with the centrifuge. In the following table the viscosity of etherized and normal control eggs is compared. The viscosity numbers represent the number of seconds of centrifugal treatment necessary to produce a given degree of granular movement. Usually the eggs were centrifuged until the clear hyaline zone extended along one third or one half of the axis of the egg. Naturally a number of tests had to be performed to obtain any given value. The centrifugal force employed was approximately 4,968 times gravity and the centrifuge used was an ordinary hand centrifuge with hæmatocrit attachment. Details of technique are given in earlier papers (16, 22). Because of the fact that a series of tests had to be performed for each value of the viscosity and because moreover the viscosity of the etherized protoplasm is not constant, the tests had to be made hurriedly. They are not extremely accurate, and it is entirely possible that the inaccuracy amounts to 10 or 20 per cent. The decided difference between unetherized and etherized eggs makes this inaccuracy of small consequence. In the table the first column gives the percentage of ether used (volume per cent. in sea-water). The second column represents the time of exposure to ether, that is to say the number of minutes elapsed after immersion in ether solution at the time the critical test was made. The third column gives the relative viscosity of the etherized eggs as compared to that of the normal control eggs shown in the fourth column. The temperature of the experiments varied from 22 to 25 degrees. It is given in the fifth column.

Per Cent. Ether.	Exposure, Minutes.	Viscosity Etherized Eggs.	Viscosity Control Eggs.	Temperature.
2.5	11	10	25	23°
2.5	4	15	25	
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8.5	20	40	23
3	3	15	30	25.3

Averaging the above values it is seen that in 2½ per cent.

ether the viscosity of the protoplasm is 53 per cent. of that of the normal control eggs in sea-water. The viscosity of the eggs in 3 per cent. ether is only 48 per cent. of that of the untreated eggs.

It must not be supposed that the viscosity of the protoplasm of etherized eggs remains constant for long periods. In the 3 per cent. solution the viscosity became lower and lower until finally a minimum was reached. Then a sudden coagulation occurred. This is shown by a series of tests on eggs in 3 per cent. ether (temp. 22.0°). When these eggs were tested after an exposure of 3 minutes it was found that centrifugal treatment for 15 seconds produced only a slight hyaline zone. The same results were obtained after 6 and 10 minutes, but after an exposure of 15 minutes, similar centrifugal treatment resulted in the formation of a hyaline zone which extended along about one third of the axis of the egg. After an exposure of 20 minutes the same treatment resulted in a hyaline zone which filled nearly half of the egg. On the other hand after 26 minutes when eggs were centrifuged for 15 seconds, although some eggs showed a hyaline zone extending through half of the egg, others showed no movement of granules at all. The protoplasm in these eggs is completely coagulated. Thus in 3 per cent. ether at 22° the liquefaction of the protoplasm is soon followed by a solidification.

The question may now be asked as to which of these two conditions represents a condition of anesthesia. In my earlier paper I had claimed that only the fluid state was anesthetic and that gelation or coagulation following ether treatment resulted only in death. But Chambers states that not the fluid but the gel state is the anesthetic condition when eggs are subjected to ether. On reading Chambers' statement I was at first led to suppose that I had been mistaken in making my claim too forcibly. I thought perhaps that although it was certain that moderately long exposure to ether after coagulation had taken place was lethal, nevertheless it might be true that Chambers had removed the eggs immediately after coagulation had begun and that in this instance the coagulative action of the ether was reversible.

The question was soon put to the test. In the experiment cited above it was pointed out that coagulation occurred after 26 minutes exposure to ether. In this same experiment some of the eggs were removed from the ether solution after an exposure of 24 minutes and placed in normal sea-water. Others were removed from the ether after an exposure of 28 minutes. Some of the eggs exposed to ether 24 minutes were inseminated, following an interval of 18 minutes after removal from the ether, and some of the eggs exposed 28 minutes were inseminated following an interval of 14 minutes after removal from ether. None of the inseminated eggs showed any signs of development. As a matter of fact both the inseminated eggs and those not exposed to sperm went through the same series of degenerative changes. All of them disintegrated by breaking up into small globules.

Thus it is obvious that following the coagulative action of ether there is no recovery. The same sort of experiment was repeated a number of times always with the same result. If eggs are to recover from ether treatment they must be removed from the ether solution some few minutes before coagulation has begun.

The discussion so far has been concerned only with conditions in unfertilized eggs. In fertilized eggs the effects of ether are even more pronounced. Let us consider a sample experiment. In the following account many details of observation are omitted.

July 22 (Temp. about 24°). Eggs were fertilized at 3.35 P.M. Fifteen minutes later, at 3.50 P.M., they were centrifuged at the usual rate for 50 seconds. No zones appeared (control unfertilized eggs showed a hyaline zone about ¼ of the distance along the egg axis after 30 seconds treatment). At 3.52 P.M. some of the fertilized eggs were placed in 2½ per cent. ether in sea-water in a glass-stoppered weighing bottle. At 3.55 P.M. a centrifugal test for 60 seconds showed only a thin streak for a hyaline zone in the control untreated fertilized eggs. At 4.27 P.M. the etherized eggs when centrifuged for 20 seconds showed a hyaline zone extending more than a third of the distance along the egg axis. At 4.31 P.M. a test of the etherized eggs for 15 seconds showed a similar zone extending about one third of the axis, and at 4.33 P.M. a 10 second test showed a hyaline zone extending through approximately one fourth of the egg.

From this experiment we can conclude that when fertilized eggs are placed in 2½ per cent. ether at a time when the viscosity of their protoplasm is at its height, the ether reduces the viscosity to less than one sixth of its original value. Another experiment of the same sort may also be cited.

July 23 (Temp. 23°). Eggs were fertilized at 10.45 A.M. At 11.00 A.M. some of the fertilized eggs were put into 2½ per cent. ether in a glass-stoppered weighing bottle, others of the same lot were centrifuged for 60 seconds. The centrifugal treatment for 60 seconds resulted in a faint indication of a hyaline zone. At 11.05 A.M. a centrifugal test of the normal fertilized eggs for 80 seconds showed a hyaline zone extending about one fourth of the egg axis. Test samples of etherized eggs were centrifuged for 5 seconds at 11.12 A.M., for 10 seconds at 11.18 A.M., and for 15 seconds at 11.23 A.M. The 5 second test showed only faint indications of a hyaline zone, the 10 second test showed the zone not very plainly, the 15 second test showed it extending along one third of the axis of the egg. Later a 10 second test, at 11.35 A.M., showed the hyaline zone extending for ¼ to ⅓ of the egg axis. This test is not as trustworthy as the earlier tests, for the protoplasm appeared to be in chunks, and it is possible that the granules were fusing together. At 11.40 P.M., a 15 second test showed the hyaline zone ⅓, ¼, or absent. Coagulation is doubtless beginning.

This experiment showed that 2½ per cent. ether may lower the protoplasmic viscosity to one sixth or even one eighth of its original value in fertilized eggs.

As with the unfertilized eggs the protoplasm of the etherized fertilized eggs becomes more and more fluid with increase in ether concentration or length of exposure until suddenly coagulation occurs. Here too the onset of coagulation results in death. If the eggs are removed from the ether in the early stages of liquefaction they resume their development, but if they are kept in ether until coagulation has occurred or is about to occur, the eggs are permanently injured and never resume development.

The result of these experiments is a direct confirmation of my older results. These findings do not agree with the statement of Chambers previously referred to. Let us consider this statement closely. He says:

"Both A. Heilbronn and Weber agree with the coagulation theory of narcosis. On the other hand, L. Heilbrunn (1920) claims that the reversible effect of 2.5 per cent. ether on the sea-urchin egg occurs only when the viscosity is diminished. With higher concentrations of ether (3 per cent. +) the increased viscosity, according to him, is irreversible. He therefore concludes that narcosis implies a diminution in viscosity of the protoplasm. My results with the micro-dissection method and by observing Brownian movement do not agree with this. In 2 per cent. ether, Brownian movement was slowed down but did not cease, and cleavage delayed but not stopped. In 2½ per cent. ether, cleavage was stopped and, both by means of the needle and by the cessation of Brownian movement, this was shown to be accompanied by a decided increase in viscosity (cf. p. 300)."

The first sentence of the above quotation is not quite true. As already pointed out Weber's ideas on ether narcosis agree exactly with mine. But this is a minor point. The essential fact is that Chambers has apparently obtained actual experimental results in direct contradiction with mine. How can this be interpreted?

In a previous paper I have already taken occasion to criticize the microdissection method for its over-great subjectivity (22), and this criticism has been supported by Heilbronn (23). But Chambers claims that his results were obtained both by microdissection and by the observation of Brownian movement. For some time I was puzzled as to how to explain the difference in his findings. Finally the idea suggested itself that Chambers in his studies of Brownian movement may have subjected the eggs to heat. It has already been shown that heat and ether act together, so that in low concentrations of ether, heat coagulation is hastened (24). Such coagulation is moreover reversible. On questioning Chambers it was found that in his experiments an arc lamp was used for illumination and no special precaution was taken to eliminate the heat factor. Here then is an explanation of the divergent results obtained by the Brownian movement method. As for Chambers' results with the microdissection method, these are probably not very trustworthy, for he was doubtless influenced by the results of his Brownian movement investigations. Another worker, Hyman, also using the dissection method, has roughly confirmed my views concerning the effects of ether on the protoplasm of sea-urchin eggs (25). Miss Hyman used ordinary steel needles instead of a microdissection apparatus.

SUMMARY.

1. Water enters etherized muscle less rapidly than normal muscle, but this does not necessarily imply a change in the properties of the plasma membrane following etherization.

2. Ether does not lower the permeability of sea-urchin eggs to water.

3. Dilute ether solutions cause a very sharp decrease in the viscosity of sea-urchin egg protoplasm, both in fertilized and unfertilized eggs. Rough quantitative measurements of this decrease are given.

4. Slightly more concentrated solutions of ether produce a coagulation which is irreversible.

5. The divergent results of Chambers find a simple explanation.

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